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# **ANNALES MEDICINAE EXPERIMENTALIS ET BIOLOGIAE FENNIAE**

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## INFLUENCE OF CORTISONE ON MITOSIS

### III

#### EFFECT OF SIMULTANEOUSLY APPLIED CORTISONE AND CELL SUSPENSIONS<sup>1</sup>

by

H. TEIR and A. ISOTALO

(Received for publication November 2, 1953)

Cortisone has an antimitotic effect upon the epidermis of the rat (1, 2, 3, 7) but not on the outer orbital gland of the same animal (2, 7). By parenteral injection of homologous tissue suspensions the mitotic ratio can be increased, for instance, in the outer orbital gland (6), the epidermis (8), the liver (9) and the stomach epithelium (4) of the rat. The purpose of the present investigation was to ascertain whether cortisone has not any effect on this stimulation of mitosis.

#### METHODS AND EXPERIMENTS

Young rats of the Wistar strain were used for the experiments. In the other respects, too, the method used in the previous experiments with cortisone was followed (7). The mitotic ratio in the outer orbital gland and in the epidermis was determined. Cell suspensions of outer orbital gland of 2-week-old rats were prepared in the same way as before (7).

In order to obtain suitable doses of tissue suspensions for 1-month-old rats, a preliminary experiment was first performed. Doses of 1, 0.5, 0.25 and 0.1 mg of orbital gland tissue suspensions

<sup>1</sup> An investigation aided by a grant from The Sigrid Jusélius Foundation and The Damon Runyon Memorial Fund for Cancer Research.

from 13-day-old rats were injected intraperitoneally into 32 rats whose weight varied between 35 and 65 g. The animals were decapitated in four groups, 5, 12, 24 and 48 hours after injection. 8 rats which received intraperitoneal injections of 1 ml. saline solution acted as controls.

The mean mitotic value for the 32 rats which received orbital gland suspension was  $17.8 \pm 1.8$ , and for the 8 controls  $9.7 \pm 1.9$ . If the rats which received suspension are grouped according to the time of influence, the following mean values with corresponding standard errors for the mitotic ratio of 8 animals of the same group are obtained:

5 hours	$11.4 \pm 1.1$
12 "	$23.6 \pm 2.8$
24 "	$26.5 \pm 5.8$
48 "	$9.6 \pm 1.2$

It is seen that the greatest effect was obtained 12 and 24 hours after injection. If, on the other hand, the rats are grouped on the basis of the quantity of tissue injected, the following mean values are obtained:

1.00 mg	$16.6 \pm 4.6$
0.50 "	$20.5 \pm 3.8$
0.25 "	$15.1 \pm 2.7$
0.10 "	$18.0 \pm 3.7$

A dose of 0.5 mg. of tissue gave the best effect, but the other doses also stimulated the mitotic activity.

The first experiment with simultaneous application of cortisone and orbital gland suspension comprised 20 26-day-old rats which received 0.15 mg of tissue from 14-day-old rats. The body weight varied between 32 and 42 g. The time of influence was 24 hours. A cortisone dose of 2.5 mg was given 4 hours before decapitation. The mean values for the mitotic activity in the outer orbital gland and the epidermis were:

	Orbital Gland	Epidermis
Orbital gland suspension .....	$17.2 \pm 3.4$	$3.0 \pm 0.6$
Orbital gland suspension + cortisone .....	$12.2 \pm 1.8$	$5.2 \pm 0.7$
Cortisone .....	$11.4 \pm 0.6$	$3.0 \pm 0.5$
Saline solution .....	$8.0 \pm 1.6$	$3.3 \pm 0.7$

With orbital gland suspension, a somewhat increased mitotic activity was observed in homologous organs but not in the heterologous epidermis. Cortisone had no antimitotic effect in this experiment. However, cortisone seemed to a certain degree to reduce the stimulating effect of the cell suspensions.

We made yet another experiment in order to verify the effect of cortisone on the mitotic stimulation obtained by injection of homologous tissue suspension. Out of 30 1-month-old rats whose weight varied between 49 and 88 g., 10 received 0.5 mg orbital gland tissue and 10 likewise tissue suspension and simultaneously 5 mg of cortisone 4 hours before decapitation. The remaining 10 rats acted as controls, receiving saline solution only. In each group 5 rats were killed 12 hours and 5 24 hours after injection of either suspension or saline solution. The mean values for the mitotic index were:

	Orbital Gland	Epidermis
Orbital gland suspension, 12 hrs .....	25.5±6.5	3.3±0.1
"    "    "    , 24 " .....	9.8±1.5	3.2±0.7
Orbital gland suspension +		
cortisone , 12 " .....	6.8±1.5	2.2±0.1
"    "    "    , 24 " .....	8.0±1.6	3.2±0.1
Saline solution , 12 " .....	12.6±1.1	5.3±0.7
"    "    "    , 24 " .....	6.6±1.0	3.4±0.3

The mitotic stimulation induced in the orbital gland by orbital gland suspension disappeared when the rats received cortisone. The mitotic ratio in the skin was not to any appreciable degree influenced by cortisone nor by the orbital gland suspension.

#### DISCUSSION

By intraperitoneal injection of tissue suspensions of outer orbital gland of 2-week-old rats, a mitotic stimulation was obtained in homologous organs but not in the heterologous epidermis of 1-month-old rats. A rather marked individual variation was seen. Such a variation must generally be reckoned with in experiments of this kind (4, 6, 8, 9), just as in colchicine experiments (5). The variation was not dependent on the body weight.

Cortisone does not directly influence the mitotic ratio in the outer orbital gland (2, 7). On the other hand, it influences the

colchicine effect insofar as, when injected 4 hours before decapitation, it reduces the colchicine mitotic index (2). In the present experiment, cortisone had a similar effect on the mitotic stimulation obtained by injection of homologous tissue suspensions. This is of interest, since it argues in favour of the assumption that cell multiplication is controlled by a peripheral humoral system on the one hand and a superior humoral system on the other (7). This correlation is particularly evident in organs whose mitotic activity is not influenced by cortisone as such, whereas it is not easily noticed in organs e.g. epidermis (1, 2, 3, 7) upon which cortisone has a direct antimitotic influence. In the present experiment however, no such effect could be established with certainty in the epidermis.

#### SUMMARY

1. Intraperitoneally injected tissue suspensions of the outer orbital gland stimulated the mitotic activity in the outer orbital gland but not in the epidermis of 1-month-old rats.

2. Cortisone neutralizes this mitotic stimulation, although it has no direct antimitotic effect on this organ. This indirect antimitotic effect of cortisone argues in favour of the assumption that the mitosis-stimulating factor activated both by tissue degeneration in vivo and by mashing of tissue in vitro is influenced by the adrenal gland.

#### REFERENCES

1. GREEN, H. N., and GHADIALLY, F. N.: *Brit. Med. J.* 1951:1:496.
2. ISOTALO, A., and TEIR, H.: *Ann. med. exper. et biol. Fenniae* 1953:31:301.
3. STUDER, A., and FREY, J. R.: *Dermatologica* 1952:104:1.
4. SUNDELL, B., and TEIR, H.: *Exptl. Cell Research*, in press.
5. TEIR, H.: *Acta pathol. et microbiol. Scand.* 1944, Suppl. 54.
6. TEIR, H.: *Acta pathol. et microbiol. Scand.* 1952:30:158.
7. TEIR, H., and ISOTALO, A.: *Ann. med. exper. et biol. Fenniae* 1953:31:171.
8. TEIR, H., KILJUNEN, A. and PUTKONEN, T.: *Ann. Chir. et Gynaec. Fenn.* 1951:40:60.
9. TEIR, H., and RAVANTI, K.: *Exptl. Cell. Research*, 1953:5:500.

## AN ANTI-LEWIS<sup>b</sup> SERUM

by

R. KOULUMIES<sup>1</sup> and O. MÄKELÄ

(Received for publication November 6, 1953)

The first anti-Lewis<sup>a</sup> serum was discovered in 1946 (1), the first anti-Lewis<sup>b</sup> in 1949 (2). Subsequently, many samples of the former have been found, whereas the latter seem to prove less numerous (3, 4, 5). That the proportion of the frequencies of occurrence should be something like this stands to reason. The relative frequency of the Le<sup>a</sup> and Le<sup>b</sup> antigens in the blood cells is approximately 13:87. Although the absence from erythrocytes of a given Lewis antigen does not necessarily involve the existence in serum of the corresponding antibody, the occurrence of an antigen will make the simultaneous appearance of the antibody impossible. That is why the proportion of the sera that may contain an anti-Le<sup>b</sup> to those which may contain an anti-Le<sup>a</sup> is ca. 13:87.

As a rule, it is not possible to detect the Lewis sera with the usual method of blood grouping, because A<sub>1</sub> and B cells used for testing purposes are but seldom agglutinated by them. Thus, for example, not a single instance has been reported in Finland.

Searching for anti-O sera, we have been able to find a serum containing extra-agglutinins. It was found in the blood of a housewife of 38 (Mrs. K.). An unusual antibody had also been discovered in her serum three years ago, but it had not been subjected to close examination. Mrs. K. has three healthy children aged 13 and 5 years, the youngest 5 months. There has been no miscarriages

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<sup>1</sup> Aided by a grant from the Sigrid Jusélius Foundation.

nor has she been given blood transfusions. Her blood group was found to be A<sub>1</sub> Rh+ABH-secretor. Agglutination, albumin, and anti-globulin tests did not indicate the presence of Rh antibodies.

In most cases O and A<sub>2</sub> cells were positively agglutinated by the serum, while a few were not agglutinated. It did not agglutinate A<sub>1</sub> cells. As far as we know, this is characteristic of anti-Lewis sera only. In the absence of recognizable Lewis sera the problem could not be solved in a direct manner. Instead two indirect methods were employed.

The agglutination of the red cells of 20 secretors and 12 non-secretors was examined with the serum of Mrs. K. The secretor characteristics had been determined with an anti-H extract obtained from *Cytisus sessilifolius* (6). It was found out that the cells of all secretors except one (Mr. H.) were agglutinated by Mrs. K's serum. The titre of the serum was defined with six specimens of O and A<sub>2</sub> erythrocytes. At 18° C it varied between 1/2 and 1/16. The cells of not a single non-secretor were agglutinated by the serum

TABLE 1

	Agglutination	No Agglutination
ABH-secretors .....	19	1
ABH-non secretors .....	0	12

. Red cell agglutination by Mrs. K's serum.

Secondly, we examined how different kinds of saliva inhibited Mrs. K's serum from agglutinating red cells sensitive to it. Of the nine saliva specimens five belonged to secretors, four to non-secretors. The serum used in the tests was undiluted so that the final dilution contained four agglutinating doses. The saliva of a secretor inhibited an agglutination in dilutions from 1/64 to 1/512, that of a non-secretor did not do so in the dilution of 1/4. (Table 2).

From these tests we can infer that some saliva specimens contain an antigen which corresponds with the extra-agglutinins of Mrs. K. Of blood group antigens being secreted into saliva, only ABH and Lewis antigens are known. The unknown antigen in question cannot be a H substance, because, in that case, all

TABLE 2

	Blood Group	Secretion of ABH-Substances	RedCell Aggl. by Mrs. K's Serum	The Dilutions of the Saliva Specimens Used									Saline Contr.
				1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	
K.	O	secre.	+	0	0	0	0	0	0	0	1	3	3
E.	O	secre.	+	0	0	0	0	0	0	0	0	1	
M.	O	secre.	+	0	0	0	0	0	0	0	2	3	
V.	O	secre.	+	0	0	0	0	0	0	1	2	2	
H.	A <sub>2</sub>	secre.	—	0	0	0	0	0	1	2	2	3	
S.	O	non-secre.	—	3	3	3	3	3	3	3	3	3	
N.	O	non-secre.	—	3	3	3	3	3	3	3	3	3	
R.	O	non-secre.	—	3	3	3	3	3	3	3	3	3	
W.	O	non-secre.	—	3	3	3	3	3	3	3	3	3	

The ability of the different saliva specimens to inhibit the agglutination by Mrs. K's serum.

Figures 1—3 indicate degree of agglutination.

0 red cells ought to be agglutinated by Mrs. K's serum. Thus the serum belongs to the Lewis system.

The Lewis blood groups correlate almost perfectly with the characteristics of ABH secretors. Not a single ABH non-secretor whose saliva or blood cells do not contain a Lewis<sup>b</sup> antigen has been hitherto found with the aid of recognized Lewis<sup>b</sup> sera. On the contrary, it has been discovered in the saliva, at least, of all ABH secretors with one or two exceptions. The occurrence of a Lewis<sup>b</sup> substance in blood cells is more irregular; in A<sub>1</sub> and A<sub>1</sub>B groups it is almost nonexistent. A<sub>2</sub>B and B are intermediate forms; in O and A<sub>2</sub> groups even the red cells produce very reliable results. Findings based on saliva are always dependable, those based on red cells can be relied upon in cases where an agglutination of the erythrocytes occurs.

As we said above, the erythrocytic antigens corresponding to Mrs. K's agglutinins correlated perfectly with secretor genes, a single case excepted. It should be noted that even in this case the saliva of the person concerned (Mr. H) was found to contain those antigens so that he, too, must be regarded as Mrs. K.-positive. Thus we are bound to consider the serum of Mrs. K. to be a pure anti-Lewis<sup>b</sup> serum.

## REFERENCES

1. MOURANT, A. E.: *Nature* 1946:158:237.
  2. ANDRESEN, P. H.: *Acta Path. Microbiol. Scand.* 1948:25:728.
  3. RACE, R. R., and SANGER, R.: *Blood Groups in Man.* Oxford 1950:193.
  4. GRUBB, R.: *Acta Path. Microbiol. Scand.* 1951:28:61.
  5. SIMMONS, R. T., and JACOBOWICZ, R.: *Med. J. Australia* 1951:vol. I:497.
  6. KOULUMIES, R.: *Ann. Med. Exper. Biol. Fenn.* 1950:28:160.
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## EFFECT OF AUREOMYCIN, VITAMIN B<sub>12</sub>, FOLIC ACID AND AMINOPTERIN ON THE METAMORPHOSIS OF TADPOLES

by

KIMMO K. MUSTAKALLIO and ANTTI TELKKÄ

(Received for publication November 18, 1953)

In a preliminary short-term experiment we could demonstrate that aureomycin causes a weight gain in tadpoles (6). Using a more extended experimental period in the present study, we intended to investigate whether the weight gain can be interpreted as an animal protein factor effect or being due to some alterations in the metamorphosis. Since the growth-promoting effect of aureomycin is probably connected to the animal protein factor effect of vitamin B<sub>12</sub> (5, 8), the effects of vitamin B<sub>12</sub>, folic acid and their antagonist, aminopterin, were also investigated.

### PRESENT INVESTIGATION

The series consisted of 400 tadpoles (*Rana temporaria*), which were collected from the same small pool on May 27, 1953. They were all about the same size (13—15 mm in length) and had no macroscopically discernible hindlegs. The series was subdivided into eight groups of fifty tadpoles, each placed in a separate aquarium of 5 litres. The groups were kept under identical conditions with regard to space, temperature, light and food, receiving daily an abundant and equal portion of fresh ground beef muscle. The food-remainings were always removed before fresh food was administered. The 5 litres of tap-water was changed every third day.

The first group served as controls. The second group was given every third day during the entire experimental period 1.5 mg of pure powdered aureomycin hydrochloride (Lederle)<sup>1</sup> per 100 cc of water. The third group received every third day 1.5  $\mu$ g of vitamin B<sub>12</sub> («Normocytin» Lederle)<sup>1</sup> per 100 cc of water. The fourth group was given every third day 70  $\mu$ g of pteroylglutamic acid («Folicid» Orion) per 100 cc of water. The fifth was every third day treated with 20  $\mu$ g of 4-aminopteroylglutamic acid («Aminopterin» Lederle) per 100 cc of water. The sixth group received every third day 1.5 mg of aureomycin plus 20  $\mu$ g of aminopterin per 100 cc of water. The seventh group was given every third day 1.5  $\mu$ g of vitamin B<sub>12</sub> plus 20  $\mu$ g of aminopterin per 100 cc of water, and the eighth group was treated with 70  $\mu$ g of folic acid plus 20  $\mu$ g of aminopterin per 100 cc of the freshly changed water.

The experiment was initiated on May 29 and ended on September 12, 1953. As the forelegs emerged from under the branchial arches — an easily ascertainable stage of metamorphosis — the tadpoles were killed with chloroform and were weighed.

#### RESULTS

The times of metamorphosis in different groups are presented in figures 1, 2, and 3. The signs in the last column show the percentage of failing metamorphosis.

During the experiment, 1 to 8 animals died in each of the groups, the highest mortality being in the aminopterin-groups. As is clear from the figures 1 and 2, no significant differences could be established in the times of metamorphosis in the control, vitamin B<sub>12</sub> and folic acid groups. The aureomycin-group and the aminopterin-group showed a retarded metamorphosis which seems to be more pronounced when aureomycin was administered together with aminopterin. Vitamin B<sub>12</sub> and folic acid had no apparent effect on the retardation induced by aminopterin.

The mean weight of the tadpoles in the control group was  $220.7 \pm 8.2$  mg, that in the aureomycin-group  $301.3 \pm 10.3$  mg, in the vitamin B<sub>12</sub>-group  $231.5 \pm 7.4$  mg, in the folic acid-group  $222.1 \pm 8.1$  mg, in the aminopterin-group  $255.8 \pm 11.6$  mg, in

<sup>1</sup> Kindly supplied by Lederle Laboratories Division, American Cyanamid Company, through the courtesy of H. Roos, M.Sc.

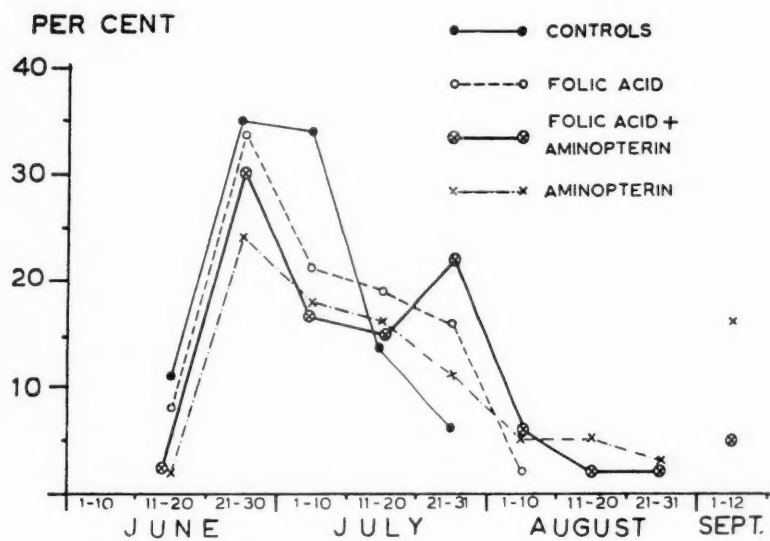


Fig. 1.

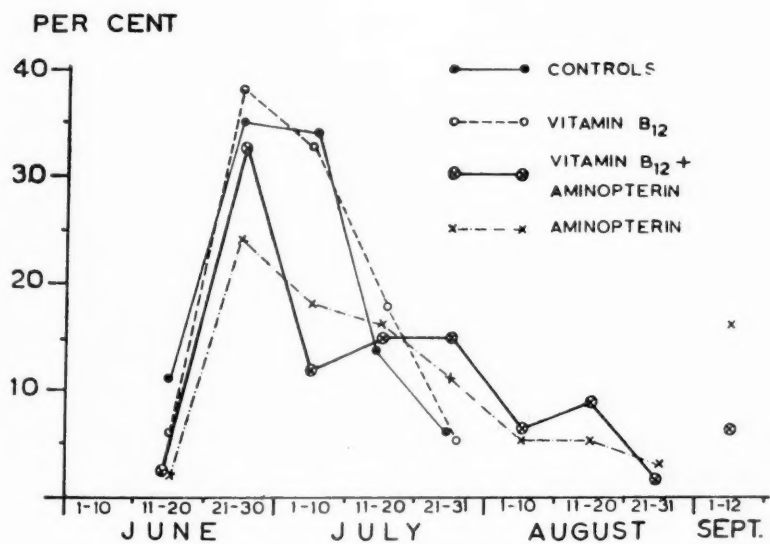


Fig. 2.

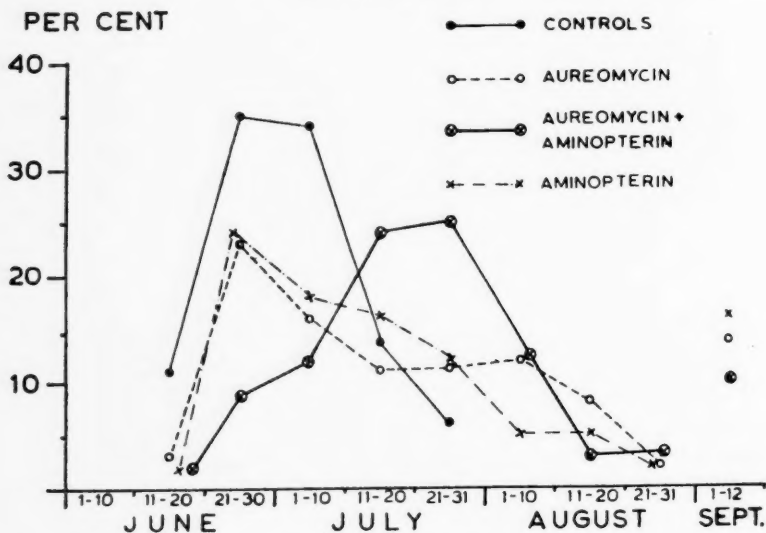


Fig. 3.

the aureomycin plus aminopterin-group  $334.5 \pm 12.4$  mg, in the vitamin B<sub>11</sub> plus aminopterin-group  $258.9 \pm 9.9$  mg and in the folic acid plus aminopterin-group  $231.5 \pm 10.2$  mg. Statistically significant were the differences between the control group and aureomycin-group, between the control group and aureomycin plus aminopterin-group, and between the aminopterin and the

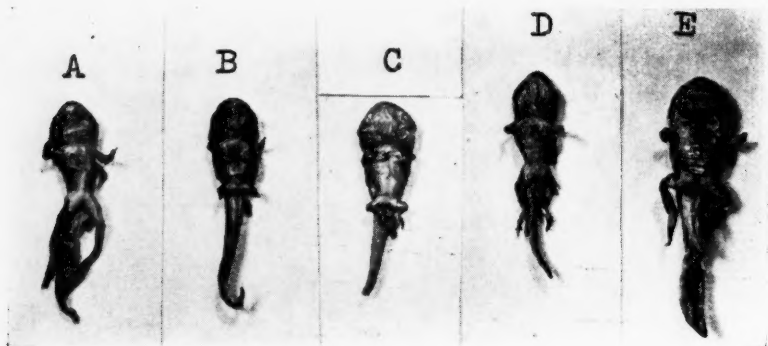


Fig. 4. — A. Control. B. Tadpole Treated with Aminopterin. C. Aminopterin plus Folic Acid. D. Aminopterin plus Vitamin B<sub>12</sub>. E. Aminopterin plus Aureomycin.

aureomycin plus aminopterin-group. Probably significant were the differences between the control group and the aminopterin-group, between the aureomycin-group and the aureomycin plus aminopterin-group, and between the folic acid and the aminopterin group.

The animals treated with aminopterin had disproportionately small legs with pronounced atrophy in the distal parts (Fig. 4). The legs were similarly affected in the folic acid plus aminopterin-group. In vitamin B<sub>12</sub> plus aminopterin-group the legs were more proportionate, but smaller than in the control group.

#### DISCUSSION

In experiments with anuran larvae the retardation in metamorphosis is generally accompanied with a gain of weight (3). Our results indicate that the retardation of metamorphosis brought about by aureomycin is the main factor in the weight gain observed in the aureomycin-treated tadpoles. It is unlikely an animal protein factor effect, since the vitamin B<sub>12</sub> had no effect on the tadpoles. The doses of aureomycin and vitamin B<sub>12</sub> were, however, comparable, because both of them administered in the same proportion to warm-blooded animals call forth a similar growth-response (1). In rats aureomycin inhibits the growth-depressing action of aminopterin (7) but in tadpoles their action was rather synergistic. Aminopterin seems to interfere with the more actively growing areas, those tissues which grow more rapidly, being most affected. This is in agreement with the observation of Rosenbaum and Velardo (4). Furthermore, aminopterin seems to have a general retardating effect on the metamorphosis. Folic acid in the concentration used, did not alter the effects of aminopterin. In experiments with warm-blooded animals, the reversibility of the effects of aminopterin by folic acid is negligible or slight (2). Vitamin B<sub>12</sub>, in the dose used, had a slight protecting action against the local growth-inhibition in the legs of the tadpoles treated with aminopterin.

## SUMMARY

Aureomycin retarded the metamorphosis of tadpoles. It seems to cause a weight gain by this way. Aminopterin induced also a retardation in the metamorphosis, which was more pronounced when aureomycin was administered together with aminopterin. Besides, aminopterin showed a local growth-depressing action in the otherwise rapidly growing legs. Vitamin B<sub>12</sub> and folic acid, in the doses used, had no apparent effect on the metamorphosis, but vitamin B<sub>12</sub> had a slight protective action against the local growth-inhibition induced by aminopterin.

## REFERENCES

- 1) CATRON, D. V., MADDOCK, H. M., SPEER, V. C., and VOHS, R. L.: Antibiotics & Chemotherapy (Wash.) 1951:1:31.
  - 2) JUKES, T. H.: Federation Proc. 1953:12:633.
  - 3) LYNN, W. G., and WACHOWSKI, H. E.: Quart. Rev. Biol. 1951:26:123.
  - 4) ROSENBAUM, R. M., and VELARDO, J. T.: Nature 1951:168:424.
  - 5) STOKSTAD, E. L. R.: Antibiotics & Chemotherapy (Wash.) 1953:3:434.
  - 6) TELKKÄ, A., and MUSTAKALLIO, K. K.: Ann. med. exper. et biol. Fenniae 1953:31:91.
  - 7) WAISMAN, H. A., GREEN, M., CRAVIOTO-MUNOZ, J., RAMENCHIK, A., and RICHMOND, J. B.: Proc. Soc. exp. Biol. Med. 1951:76:384.
  - 8) ZINK, A.: Internat. Rev. of Vitamin-Research 1952:23:471.
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## FLAVASPIDIC ACID AS AN ANTHELMINTIC

by

V. M. ANTONEN

(Received for publication November 20, 1953)

It has appeared to be difficult to find out a drug against the tapeworms, whose therapeutic doses would be effective enough and, at the same time, causing no untoward reactions in the host.

Pumpkin seeds (*semina cucurbitae*), due to their harmless nature, have been used against the tapeworms, especially to patients in poor general condition. There are no exact data on their effectiveness, and even they involve some danger (4). It has been reported that kamala (10), thymol (19), and atebirin (15) cause hardly any side-effects. Nevertheless, their use has remained relatively rare in Finland compared with that of male fern extracts.

Male fern extracts are, however, not excellent anthelmintics, as the effectiveness and toxicity of their therapeutic doses are so close to each other that there have occurred cases of poisoning (18). Male fern extracts are extremely dangerous when administered to patients with nephritis, arteriosclerosis, or heart disease (7, 10).

The toxicity of different male fern extracts varies considerably, hence, even small doses may prove fatal (10, 18). Besides, it has been difficult to determine the effectiveness and toxicity of the extract, owing to the fact that there exist no generally accepted exact methods of standardizing (1, 18).

That the toxicity of male fern extracts varies in various animals (11, 20, 22) makes it complicated to evaluate even the biological

methods of standardizing. Huhtala (11) has noted that there exists a certain correlation between therapeutic effectiveness and bactericidal activity of filix extracts, *i.e.*, the strongly bactericidal extracts also have strong therapeutic effectiveness.

According to Widén (20) and Huhtala (11), all effective male fern extracts contain *e.g.* flavaspidic acid. It has been estimated that crude magnesium filicin (*Dryopteris filix-mas*) contains 11—18 per cent (8), 6—10 per cent (2), or 6.6—8.8 per cent (13) flavaspidic acid. According to Huhtala (11), *Dryopteris filix-mas* extract contains 1.96 per cent flavaspidic acid, and Widén (20) estimates the acid contents generally to 2—5 per cent, but it may occasionally amount to 40 per cent. Mühlemann (14), using his own method, has found that crude magnesium filicin contains 28 per cent flavaspidic acid. He considers it as the effective compound of male fern extract, the other filix substances being of less therapeutic value.

Huhtala (11), too, has pointed out the biological effectiveness of flavaspidic acid: the male fern extracts, which contained large amounts of flavaspidic acid, had stronger bactericidal influence. Flavaspidic acid has proved less poisonous than *e.g.* filmaron or extractum filicis (1). According to Straub (16), it is less poisonous than aspidin. Toft (17) and Huhtala (11) have, on the other hand, paid attention to its remarkable hemolytic capacity. Besides, it is strongly poisonous to heart and cardiovascular system, but, fortunately, its resorption from gastro-intestinal tract is poor (12).

#### WRITER'S INVESTIGATIONS

I have found no references in the literature as for the clinical use of flavaspidic acid against tapeworms, though it has proved biologically very effective. Its low toxicity justified a clinical trial, and it has been used in the Municipal Hospital for Internal Diseases in Kuopio since spring, 1953.<sup>1</sup>

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<sup>1</sup> Flavaspidic acid was provided to me by Mr. E. Aho, who isolated it in The Pharmaceutical Manufacturers, Leiras, Turku, Finland. To him and to Professor P. Brummer, M.D., who have made my investigations possible, I wish to express my sincere thanks.

## THERAPY

The patients were allowed to take a light meal in the afternoon of the day preceding that on which therapy was begun. The anthelmintic was administered with water or coffee into the empty bowel within half an hour's time. Two hours after the administration of the drug the patients were given 25 g magnesium sulphate in water solution, and two hours later they were given an enema.

The results will be seen from Table 1, which also includes results of some earlier investigations.

TABLE 1

Investigator	Anthelmintic	Number of Cases	Positive Number	Positive %	Side-effects
Grönberg .....	Extr. filicis			68—80	
Grönberg .....	Kamala			88	38
Huhtala .....	Extr. filicis (2.5—4.0 g)	152	132	87	30
Vartiainen, O. ..	Extr. filicis (3.0—3.5 g)	100	100	100	
Vartiainen, O. ..	Extr. filicis (follow-up)	100	92	92	
Vartiainen, O. ..	Thymol	178	156	89	< 4
Vartiainen, O. ..	Thymol (follow-up)	169	83	49	< 4
Ruikka .....	Atebrin	50	38	76	4
Writer's .....	Extr. filicis (2.5—4.0 g)	85	64	75	
Investigations	Filmarex (Leiras)	13	8	ca 60	
	Semina cucurbitae	17	11	ca 60	
	Flavaspidic acid	76	65	86	10

Grönberg (6) considered the therapy successful, if the head of the worm had been identified in the stools. According to Huhtala (11), the result was positive if the worm came out with its head, or, if no worm eggs were discovered in microscopic examinations four weeks or later after the therapy. Vartiainen (19) considered the therapy successful, if the worm or parts of it came out, or if no worm eggs were discovered in microscopic examinations in follow-up cases one to eight weeks after the administration of the

drug. In Ruikka's (15) investigations the positive number means cases in which the worm or parts of it were identified in the stools. I have considered the therapy successful, if the worm or parts of it have come out, no matter if the head has been identified or not. Only in the cases, in which the patient stayed in the hospital for several weeks, the stools were daily examined microscopically. Due to the great number of reinfections, especially in areas where the incidence of tapeworm infestation is high, as e.g. in the eastern parts of Finland, the follow-up investigations, carried out in out-patients' department, are of less significance.

The criterion used has proved useful when determining the relative effectiveness of various anthelmintic drugs, though the exact effectiveness of anthelmintic therapy is very difficult to determine. So, e.g., Fischer (5) and Wigand (21) remark that the identification of the head of worm in the stools need not exactly mean that the therapy has been successful, as the patient may often have several parasites at the same time. Further, the failure to identify the head (5) or parts of worm (3) need not absolutely indicate that the therapy has been a failure (5), as it may happen that the worm becomes digested in the gastro-intestinal tract (3). In Fischer's investigations the head was identified in 22 cases, though, according to the follow-up investigations, the therapy proved successful in 42 cases (5).

Discrediting the value of stool examinations Huhtala (11) points out that even after the unsuccessful therapy the worm may not be able to release eggs during the next four weeks. Accordingly, the follow-up investigations should be carried out after this period. In my opinion, the eggs may be missed in microscopic examinations even in positive tapeworm cases.

Against this background the results of anthelmintic therapy, obtained by various writers, are not comparable. The discrepancies are still augmented by the fact that the various male fern extracts may greatly differ *inter se* in effectiveness. E.g., the extr. filicis used by Vartiainen has apparently been more effective than that used in my investigations. Table 1 shows further that flavaspidic acid has been more effective anthelmintic drug than the extr. filicis capsules I have used.

## CLINICAL CASES

The anthelmintic therapy with flavaspidic acid was applied to 76 patients, whose age will be seen from the following Table 2.

TABLE 2

Age in Years	14—29	30—49	50—69	70—79
No. of Patients	7	23	39	7

Several of the patients were of advanced age and most of them were suffering from a complicating disease as Table 3 shows.

TABLE 3

Complicating Disease	No. of Patients
Arterial hypertension, myocardial degeneration . . . . .	25
Congestive heart failure . . . . .	10
Coronary insufficiency (St. post infarct. cordis) . . . . .	4
Bronchial asthma . . . . .	2
Abscess of the lung . . . . .	1
Diabetes mellitus . . . . .	5
Acromegalia . . . . .	1
Cancer of the stomach . . . . .	2
Hepatopathy . . . . .	4
Nephropathy . . . . .	3
Anemia hyperchromica . . . . .	7
Anemia hypochromica . . . . .	4
Nihil . . . . .	8

TABLE 4

Flavaspidic Acid Dosage in Grammes	Women		Men	
	Positive	Negative	Positive	Negative
1.5	—	—	1	—
1.0	6	1	16	4
0.83	7	—	3	—
0.67	26	4	2	2
0.5	1	—	3	—
Total	40	5	25	6

The dosage of flavaspidic acid has been of experimental nature. In accordance with the biological experiments a single dose did not exceed 1.5 g and varied from 0.5 to 1.5 g. Table 4 records the dosages used and their results.

Seven cases infested with *Taenia saginata* were all responding to flavaspidic acid therapy. The dosage was as follows:

- 1.5 g in one case
- 1.0 g in three cases
- 0.83 g in one case
- 0.67 g in two cases

In 69 patients with *Diphyllobothrium latum* the therapy was successful in 58 cases. Seven of the cases in which the worm did not come out were checked daily for worm eggs:

In four cases (flavaspidic acid 1.0 g) no worm eggs were discovered.

In two cases (flavaspidic acid 0.67 g) the eggs were discovered only during the first two weeks after the therapy. The other patient of this group was given a new dose of flavaspidic acid (1.0 g) after two months with positive results.

In one case (flavaspidic acid 0.67 g) worm eggs were discovered continually in follow-up investigations, and the therapy (flavaspidic acid 1.0 g) was repeated after three months. No parts of the worm were identified in the stools, but the microscopic examinations for worm eggs yielded negative results during the next two months.

It is evident, hence, that 1.0 g of flavaspidic acid is very effective dose, as it seems to kill the worm, which then becomes digested and cannot be identified in the stools, not even after the successful therapy. Table 4 shows further that as small a dose as 0.5 g can be strong enough to expel the worm.

In order to detect any side-effects the following examinations were carried out before the administration of the anthelmintic drug and seven to 24 hours (generally 24 hours) after it (Table 5).

As will be seen from Table 5, flavaspidic acid produced only a few side-effects, though most of the patients were suffering from a complicating disease. During or after the therapy the side-effects were discovered in the following cases.

TABLE 5

Examination	Number of Cases		Pathological Changes due to Therapy
	Before Therapy	Before and after Therapy	
Physical Examination .....	76	76	3 (Cases 3, 4, and 5)
Sedimentation Rate .....	74	45	2 (Cases 3 and 4)
Hemoglobin Content .....	71	33	—
Blood Count .....	71	33	—
Icterus Index (Meulengracht) ..	57	47	2 (Cases 3 and 5)
Serum Flocculation Test (Takata-Ara) .....	54	47	—
Stolte Test .....	58	47	—
Thymol Turbidity Test (Mactagan) .....	7	7	—
van den Bergh's Test (Direct and Indirect) .....	24	22	—
Urine Bilirubin (Rosin-Trousseau Iodine Test) .....	56	48	—
Urine Urobilinogen (Ehrlich Test)	56	48	—
Urine Urobilin (Schlesinger Test)	56	48	—
Urine Albumin .....	72	30	—
Urine Sugar .....	72	30	—
Urine Sediment .....	10	9	—
Electrocardiogram .....	65	38	3 (Cases 6, 7, and 8)

*Case 1.* — A man, aged 51, had after extr. filicis therapy (3.5 g), in 1925, turned yellow and sick. Liver function tests yielded negative results before the administration of flavaspidic acid (1.0 g). About two hours after the administration of the drug the patient complained of nausea and severe epigastric pain. However, the symptoms disappeared spontaneously in half an hour. The patient's general condition was good. An electrocardiogram and liver function tests showed no pathological changes.

*Case 2.* — A woman, aged 56, had been suffering from congestive heart failure for five years. Two hours after the administration of flavaspidic acid (0.67 g) she began to complain of nausea and epigastric pain. The symptoms disappeared spontaneously in an hour. An electrocardiogram and laboratory examinations showed no abnormalities. No changes were noted in the blood pressure, and heart failure had not worsened.

*Case 3.* — A woman, aged 54, had taken 3.5 g of extr. filicis at home two years ago and «was about to die». In May 1953, liver was discovered to have been expanded, but the icterus index, the Takata-Ara test, and the Stolte test were normal. The Rosin-Trousseau iodine test, the Ehrlich

test, and the Schlesinger test yielded negative results. The patient was administered flavaspidic acid (0.67 g). The therapy caused no untoward reactions, but it proved a failure. The repeated dose of flavaspidic acid (1.0 g) after to months was successful. The patient's general condition was good before the therapy, liver being slightly expanded. Laboratory examinations showed similar results as in May 1953, except that the sedimentation rate was 15 mm in an hour and the direct van den Bergh's test showed negative, the indirect positive results. The electrocardiogram was normal.

The patient complained of weakness and nausea and turned pale and perspired about an hour and a half after the administration of the drug. The blood pressure fell from 130/90 to 105/60 mm Hg. The pulse was small, hardly palpable, and the legs were cold and bluish. The vomiting patient was given a lavage and oxygen. Coramine-adenosine and adrenalin were administered, and the collapse passed off in an hour and a half. The electrocardiogram was normal. The scleras were discovered icteric on the following day, and the icterus index was 1: 17. The sedimentation rate was 22 mm in an hour and the hemoglobin 96. The blood picture was as follows: erythrocytes 4,065 mill., index 1.18, and leukocytes 3950. The Stolte test was 1.90 ml and prothrombin index 110. The Takata-Ara test and the direct van den Bergh's test yielded negative results, the indirect van den Bergh's test being positive. There were no pathological changes in the urine. General condition remained good, and the patient was removed from the hospital after two days, the icterus index being then 1: 10. The scleras were no more yellow.

*Case 4.* — A woman, aged 66, has been suffering from diabetes mellitus since 1926 and from high blood pressure (ad 270/130 mm Hg) for the last 15 years. In 1949 she had infarctus cordis, and for the last four years she has suffered from congestive heart failure. In spring 1953, the patient had a severe attack of nausea, and eggs of *Diphyllbothrium latum* were discovered in the stools. In June 1953, she was administered flavaspidic acid (0.67 g). The therapy caused no untoward symptoms, but the worm was not identified in the stools and worm eggs were found in microscopic examinations continually. The patient still suffered from nausea, apparently not due to diabetes. She was administered a 1.0 g dose of flavaspidic acid in August 1953. The worm was not identified in the stools, but no worm eggs were found in the follow-up investigations during the next two months. On the following morning after the administration of the drug the patient felt dizzy and found difficulties in talking and seeing. The left leg and arm were numb. The blood pressure had fallen from 190/110 mm Hg to 160/100 mm Hg. The electrocardiogram was not changed due to the treatment. The sedimentation rate was before the therapy 35 mm in an hour, on the following day 39 mm in an hour, and a week later 45 mm in an hour. The other laboratory examinations showed no pathological changes due to the therapy. The symptoms of coronary insufficiency were not increased. Within two months the patient was completely recovered.

*Case 5.* — A man, aged 25. *Taenia saginata* had been identified in the stools for several years. When admitted to the hospital, the patient was in good condition, and the physical examinations showed no abnormalities. Therefore, he was administered a dose of 1.5 g of flavaspidic acid before consulting the results of the laboratory examinations. The icterus index was, however, 1: 15 before the therapy, the Stolte test 1.74 ml, the Takata-Ara test + 1/64, and the thymol turbidity test yielded negative values. No bilirubin was discovered in the urine, but the Ehrlich test and the Schlesinger test showed positive results. The patient was feeling well during the therapy, but the scleras were discovered icteric on the following day, and the icterus index was 1: 22. The Stolte test was 1.97 ml. The patient was administered glucose, vitamins B<sub>1</sub> and B<sub>12</sub>, and methionine. His general condition remained good, and the icteric signs disappeared in two days, icterus index being then 1: 9, the Stolte test 1.62 ml, and thymol turbidity test negative. Bilirubin, urobilin, or urobilinogen were not identified in the urine.

*Case 6.* — A woman, aged 56, was admitted to the hospital for sub-acute pyelocystitis (*Klebsiella pneumoniae*) and hypochromic anemia (hemoglobin 49). The electrocardiogram, taken before the anthelmintic therapy, showed that in leads II, III, and CF<sub>4</sub> S-T intervals took off below the zero level. The configuration of QRS in lead III was notched and split. The electrocardiographic examination, taken on the following day after the therapy (0.67 g of flavaspidic acid), showed distinctly further depression of S-T interval in lead CF<sub>4</sub>, though hemoglobin was 54.

*Case 7.* — A man, aged 46, had been suffering from coronary insufficiency for four years. In July 1953, he had heart infarct and the disease worsened. Even a light meal caused painful attack, and the electrocardiographic examination (three extremity leads, one chest lead) showed that S-T intervals were markedly taking off below the zero level. The roentgenological examinations of the oesophagus and stomach yielded negative results. The passage was normal as well as cholecystography. The patient was administered a dose of 0.67 g of flavaspidic acid. No side-effects appeared during the therapy, but the electrocardiographic examination, taken on the following day, showed that S-T intervals were more markedly depressed in the second extremity lead and chest lead.

*Case 8.* — A woman, aged 59, had been suffering from diabetes mellitus, hypertonia, and congestive heart failure for six years. The patient showed symptoms of coronary insufficiency and extrasystoles. The blood pressure was 190/110 mm Hg before the anthelmintic therapy (0.67 g of flavaspidic acid). The electrocardiographic examination showed that S-T intervals were markedly taking off below the zero level in extremity leads and elevated in chest lead. During the therapy the patient complained of slight nausea. There were no changes in the blood pressure on the following day, neither were the subjective symptoms of coronary insufficiency increased, but S-T intervals were more markedly depressed in extremity

leads and close to the zero level in chest lead. No changes were discovered in the blood sugar. The patient was removed from the hospital two days after the therapy. Her general condition was good, and, subjectively, she had no heart troubles.

#### DISCUSSION

Among the untoward reactions noted in the course or after the therapy hepatopathy was identified in Cases 3 and 5 and suspected in case 1. In cases 2, 4, and 8 it could not be excluded, however, hepatopathy was in these patients probably due to congestive heart failure and, besides, in the Cases No. 4 and 8 due to diabetes mellitus. Cases 3 and 4 were, moreover, exceptional in the respect that 0.67 g of flavaspidic acid caused no untoward reactions, while the repeated therapy (1.0 g of flavaspidic acid) caused dangerous side-effects. It remains to be solved if there was in question a mere incidence, toxicity of the dose, or hypersensitivity to flavaspidic acid.

The electrocardiographic changes in cases 6, 7, and 8 indicate that already 0.67 g of flavaspidic acid may increase the symptoms of coronary insufficiency. Due to my investigations, the maximal dose of flavaspidic acid should be no more than 0.5 g to patients with severe coronary insufficiency or suspected hepatopathy, or to patients who have not been tolerant of male fern extracts. Often this dose may already be strong enough to expel the worm. If the therapy proves a failure, it should not be repeated with larger doses of flavaspidic acid. In general, I consider it unnecessary to administer larger single doses than 0.8 g, since it has proved effective enough. By using these doses there is no danger as for the hemolytic power of flavaspidic acid, as one might expect on the basis of the experiments carried out by Toft (17) and Huhtala (11).

Flavaspidic acid is, in my opinion, valuable drug against the fish tapeworm. Its usefulness is due to the fact that its dosage is exact, because flavaspidic acid is a well defined chemical compound. Good anthelmintic effectiveness and relatively low toxicity are its other profits. Flavaspidic acid seems to be effective also against the beef tapeworm, which is generally difficult to expel.

## SUMMARY

Flavaspidic acid was administered to 76 patients against the tapeworms in varying doses (0.5 g to 1.5. g). The therapy proved successful in all seven cases with *Taenia saginata*, and in 58 cases with *Diphyllobothrium latum*. Side-effects were noted in ten per cent of the patients. Special attention ought to be paid to the patients with hepatopathy, coronary insufficiency, or to the patients which have not been tolerant of male fern extracts. The writer recommends single doses of 0.5 to 0.8 g of flavaspidic acid as an harmless and effective anthelmintic therapy.

## REFERENCES

1. AHO, E.: Suomen Apteekkariyhdistyksen Aikakauslehti. 1953:12:353.
2. BOEHM, R.: Arch. f. exper. Path. u. Pharmacol. 1897:38:35.
3. EHRSTRÖM, R.: Fabers Nordisk Laerbok: Intern Medicin. København 1945.
4. ESKOLA, O.: Duodecim 1937:53:417.
5. FISCHER, T.: Om behandlingen af bennikemask med ormbunkerot-extrakt. Stockholm 1904.
6. GRÖNBERG, J.: Farmaceut. Notisbl. 1921:30:57.
7. HALONEN, P. I., and KOSKIMIES, A.: Cardiologia 1950:17:1.
8. HAUSMANN, A.: Arch. d. Pharm. 1899:237:544.
9. HUHTALA, A.: Suomen Lääkärilehti 1946:252.
10. HUHTALA, A.: Duodecim 1948:64:640.
11. HUHTALA, A.: Vergleichende pharmakologische und chemische Untersuchungen über Farnextrakte. Diss. Vaasa 1949.
12. HUHTALA, A.: Personal communication.
13. MAIZITE, J.: According to AHO, E.
14. MÜHLEMANN, H.: Pharm. Acta Helv. 1943:8—9:1.
15. RUIKKA, I.: Duodecim 1951:67:254.
16. STRAUB, W.: According to HUHTALA, A.
17. TOFT, H.-I.: Extractum Filicis. Kemisk og biologisk Vundering. Diss. København 1946.
18. VARTIAINEN, A.: Report to the Finnish Medical Board.
19. VARTIAINEN, O.: The Anthelmintic Effects of Thymol and *p*-Cymene. Diss. Helsinki 1950.
20. WIDÉN, B.: Untersuchungen über die Phloroglucinderivate finnischer Farnarten. Diss. Helsinki 1944.
21. WIGAND, R.: Therapie der Infektionen des Menschen durch Würmer in Mitteleuropa. Leipzig 1943.
22. YAGI, S.: Ztschr. f. d. ges. exper. Med. 1914:3:64.

## VARIOLA VACCINATION AS AN ACTIVATOR OF TUBERCULOUS INFECTION

by

OLE WASZ-HÖCKERT

(Received for publication November 24, 1953)

At the time when discussion about the postvaccinal encephalitis was of current interest, Blacher (2) reported a case of a boy aged four years, who revealed meningitic symptoms four days after variola vaccination, and died of tuberculous meningitis four weeks later. He also reported a case of a girl, aged 11 years, whose primary old tuberculosis was activated after variola vaccination. Ainger (1) reported two similar cases of children, in which miliary tuberculosis with tuberculous meningitis broke out a few days after variola vaccination causing the death of the children four resp. six weeks later. Diagnoses were verified by autopsies.

In connection with general vaccination in Glasgow, Keers and Steen (3) paid attention to the fact that there appeared fresh roentgenological changes (pleuritis, caverns) in four young relatively healthy individuals with former tuberculosis. Stone (4), on the other hand, minimized the risk of vaccination pointing out that in a sanatorium only one of the 337 vaccinated patients showed slight progress in the pulmonary tuberculosis.

Apparently, vaccination does not, as a rule, affect the clinical course of the patients with tuberculosis, but there certainly are individuals in which the labile balance between the disease and the host can be unfavourably influenced by a vaccination.

## OWN CLINICAL CASE

A boy (Record N:o 2278/49, Children's Clinic, University of Helsinki), aged two years, had always been healthy, and never tested with tuberculin nor vaccinated with BCG. His father had been treated for tuberculosis in a sanatorium, and had been considered recovered since 1946.

Variola vaccination was made May 31, 1949 (0.1 cc of a 1 : 200 dilution intradermally). Moro's test, made on the same day, was positive. Three days later the temperature rose to 38.0° C, and the patient started vomiting. The meningitic symptoms and fever continued, and the boy became somnolent June 20.

The unconscious patient was admitted into the Children's Clinic June 24. After sulphathiazol treatment there appeared erythema nodosum. Mantoux test (0.01 mg) appeared to be positive. In spite of streptomycin treatment the patient died June 29, *i.e.*, four weeks after vaccination.

Post-mortem diagnosis (E. K. Ahvenainen, M.D.): Caseatio lymphonoduli pulm. et mediast. Tub. miliaria pulm., hepatitis et renis. Meningitis basalis tub.

## PRELIMINARY EXPERIMENTAL INVESTIGATIONS

The first preliminary experiment on four guinea pigs was made in the Department of Serology and Bacteriology to elucidate the clinical observations. Four tuberculin negative guinea pigs, each weighing about 800 g, were inoculated subcutaneously over the sternum each with 0.1 mg of tubercle bacilli, H<sub>37</sub>Rv strain. A week later, two guinea pigs were vaccinated intradermally with 0.1 cc variola vaccine (N:o 31/53) of a 1 : 200 dilution. The four guinea pigs were killed 41 days after the TB inoculation. The *macroscopic findings* in necropsy are depicted as follows: (Fig. 1).

*Histological samples* were taken from spleen, liver, and lungs of each animal. The examiner (Dr. L. Hjelt) had no knowledge of the origin of the samples. The results are as follows:

In the guinea pigs N:o 990 and 738 the tuberculous lesions of the spleen were considerably larger in size than in N:o 739 and 740.

**Liver:** All guinea pigs showed marked tuberculous changes, those of N:o 990 and 738 being, however, considerably larger in size and more severe.

**Lungs:** Only N:o 990 showed tuberculous lesions, the other animals were normal.

The guinea pigs N:o 990 and 738 were the vaccinated ones.

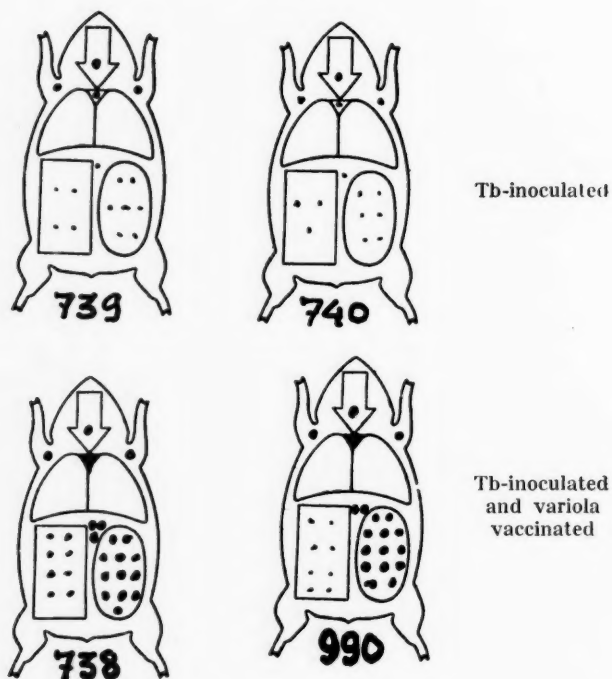


Fig. 1

The weight curves of the guinea pigs were followed during the experiment. During the last two weeks, there occurred more loss in weight of the vaccinated animals than in the control ones. This difference might be due to the small number of animals. The vaccinated guinea pigs showed a slightly positive *tuberculin test* (Mantoux, 1.0 mg) 13 days before necropsy, while the reaction of the animals not vaccinated still remained negative. Both groups reacted positively six days before necropsy.

The similar differences between the vaccinated and non-vaccinated guinea pigs, both groups inoculated previously with  $H_{37}Rv$ , also appeared in a later preliminary experiment made on 10 guinea pigs in each group (5).

#### DISCUSSION

The occurrence of tuberculous meningitis in apparently healthy individuals after variola vaccination, as reported above, can

hardly be considered to be due to a mere statistical coincidence. I have presented my preliminary report, since the observation of this phenomenon has generally been neglected, and since it has not been experimentally established with certainty. The activation of a latent tuberculous infection through variola vaccination might be explained as a result of an additional stress caused by vaccination.

#### SUMMARY

The writer reports a case in which a seemingly healthy two-year-old boy was seized with fever and meningitic symptoms three days after variola vaccination. The patient died of miliary tuberculosis and tuberculous meningitis four weeks later. The writer attempts to elucidate this observation experimentally: The four guinea pigs were inoculated with H<sub>37</sub>Rv, two of them received variola vaccination a week later. All were killed six weeks after inoculation. The necropsy as well as the histological examination revealed considerably larger tuberculous lesions in spleen and liver of the vaccinated animals than of the control ones. Similar results were obtained later on 10 guinea pigs in both groups (5).

The writer considers the phenomenon to be due to a competition of the defense mechanism of the animal against tuberculosis and vaccination.

#### REFERENCES

1. AINGER, A.: *Wien. Med. Wschr.* 1937:87:441.
  2. BLACHER, W.: *Jahrb. Kinderheilk.* 1931:130:201.
  3. KEERS, R. Y., and STEEN, P.: *Brit. J. Tuberc.* 1943:37:111.
  4. STONE, R. E.: *Amer. Rev. Tuberc.* 1931:23:706.
  5. WASZ-HÖCKERT, O., and BACKMAN, A.: To be published.
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## CORTICOTROPIN AND CORTISONE

### INFLUENCE ON EXPERIMENTAL IMMUNO-HEMOLYTIC ANEMIA IN GUINEA PIGS

by

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Although curative in many cases of acquired hemolytic anemia, splenectomy sometimes gives disappointing results (34). Hence Dameshek *et al.* (5) tested treatment with corticotropin (ACTH) or cortisone which they found to be highly effective. Favorable results have been reported also by other authors (2, 11, 4, 21), and the hormonal treatment of the disease is now well established, even if complete cure cannot be expected in all cases.

The mode of action of corticotropin and cortisone in acquired hemolytic anemia is not, however, completely understood. We must assume either that the adrenal steroids interfere with the antigen-antibody mechanism or that the hormones protect the body from the effects of that mechanism. Four phases are distinguished in the immuno-hemolytic process, i.e. 1) the antibody production, 2) the union of antigen (red cells) and antibody, 3) the direct hemolytic action of the antibody (plus complement) and 4) the destroying effect of the living organism on sensitized red cells, i.e. cells coated with antibody. Theoretically, the adrenal hormones may be thought to interfere with any of these phases. In addition they may help the organism to overcome the deleterious effect of the red cell destruction by stimulating the erythropoiesis.

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<sup>2</sup> R.T. (Canada).

The possible effects of the hormones on the different phases of the immuno-hemolytic process *in vivo* will be considered separately:

1) We know that corticotropin and cortisone cause atrophy of the lymphatic tissue and lymphocytopenia in the blood (6, 35). Hence, if the antibodies are produced by the lymphatic tissue (15), we may expect the hormones to have a suppressing effect on antibody formation. It is more difficult to predict the effect of adrenal hormones on the formation of antibodies if they are produced by the plasma cells, which now seems to be very probable (10, 25). Moeschlin and his associates found direct microscopical evidence of an altered plasma cell reaction to antigen stimulus if the animals had been pre-treated with corticotropin or cortisone (25).

Many investigations on the effect of adrenal steroids on the antibody titer have been carried out, but the results are contradictory. Dougherty, White and Chase (7) found that an injection of adrenal cortical extract into a rabbit, hyperimmunized against sheep red cells, caused a rise in antibody titer. They also found a stimulating effect on the antibody production if, during the period of immunization, the rabbits were treated with adrenal steroids (3). The first mentioned finding has been confirmed by Hammond and Novak (14), but no such effect was found by de Vries (32), if the rabbits were immunized against egg albumin. Other workers (29, 16) report an actual fall in antibody titer, caused by adrenal cortical hormones given after the period of immunization. The stimulating effect on antibody production, observed by Dougherty and associates, has not been confirmed by most investigators. Some of them report no effect whatsoever of adrenal steroids on antibody formation (20, 9, 30, 18), but several workers have found a depressing effect on the antibody production (12, 8). Dows (8), for instance, found a 50 per cent lower hemolysin titer in rats injected with rabbit cells if the rats were simultaneously treated with cortisone. Even if different authors report different results, it may be stated that adrenal steroids seem to have a depressing effect on the antibody formation, at least in some cases. The varying experimental results may be due to the antigen used, the species of animal immunized, and other circumstances. According to Moeschlin et al. (24) the antibody production is

suppressed only if the cortisone or ACTH treatment is started before the administration of antigen.

2) It has not been proved whether corticotropin and cortisone have any effect on the antigen-antibody union, but it does not seem probable that the hormones affect this phase of the immunologic reaction (19).

3) An antihemolytic effect of adrenal steroids might be predicted if the hormones reduce the cellular permeability. Menkin (23) found adrenal cortical extract to affect the capillary endothelium in inflammatory tissue in this manner. Megel and Gordon (22), on the other hand, found adrenalectomy of experimental animals to reduce the hypotonic fragility of the red cells, and Clearkin (4) did not notice any effect of cortisone on the titer of hemolytic immune serum *in vitro*. Hence we have no evidence of a direct anti-hemolytic effect of adrenocortical hormones.

4) When red cells become coated with antibody, there is probably a direct destroying effect of the antibody and complement on the cell in some cases, but in addition the living organism takes part in the destruction of the coated cells (33). Some of these cells are probably ingested by phagocytosing cells of various organs, e.g. the spleen (1). Other cells may be agglutinated, sequestered, and lysed by humoral mechanisms (13), and the adrenal steroids may to some extent affect the last mentioned mechanism by reducing the size of the most important sequestering organ, the spleen. The effect of the corticoids on the phagocytosis is still doubtful. A possible suppressing effect (26) on the phagocytosis might be an explanation of the decreased red cell destruction, but the result would, of course, be an increased red cell destruction if the phagocytosis is stimulated (28, 31).

Corticotropin and cortisone probably have a stimulating effect on the erythropoiesis (31), and even if the antigen-antibody mechanism is not affected by the hormones, they may thus help the organism to compensate for the increased red cell destruction. An increased red cell production during the treatment of acquired hemolytic anemia with corticotropin has been observed (5), but this mechanism is probably only of minor importance, because the remission during prolonged treatment is accompanied by both reduced red cell destruction and decreased reticulocytosis.

As already mentioned, it seems to have been revealed that

corticotropin and cortisone suppress the antibody formation at least under certain conditions. Hence this mechanism may explain the beneficial effect of the hormones in acquired hemolytic anemia. It is feasible, however, that these substances act on other phases of the immuno-hemolytic mechanism as well. The present work was done to clarify the last mentioned possibility. Experimental immuno-hemolytic anemia was produced in guinea pigs, and parallel experiments were run with some corticotropin treated guinea pigs, and some treated with cortisone, and some untreated, except for the immune serum injections.

An experiment of this type has been reported by Palmer et al. (27) who found a suppression of the hemolysis caused by immune serum injected into rats, if the antibody dose was small, but no effect on the hemolysis if the dose was high. In his very small experimental series, Clearkin (4) observed no effect of cortisone on experimental hemolytic anemia in guinea pigs.

#### METHODS

Anti-red cell serum, active against guinea pig red cells, was prepared by repeated intravenous injections of washed guinea pig red cells into a rabbit. The hemolysin titer of the serum was determined by making up a two-fold dilution series of the inactivated serum and adding 0.2 cc of guinea pig complement and 0.2 cc of a 2% suspension of washed guinea pig red cells to 0.2 cc of the diluted immune serum in each tube. A parallel series with tubes containing saline instead of complement was run for the agglutinin titer. All tubes were incubated for one hour at 37 C. and the titers read macroscopically, the agglutinin titer being checked microscopically. The following titers were read: complete hemolysis 1:48, end-point hemolysis 1:6144 and agglutination 1:6144, all figures expressing the final dilutions in the tubes. Hence the undiluted serum contained 16 hemolytic units per 0.1 cc if one hemolytic unit is defined as the amount of serum capable to produce complete hemolysis of 0.1 cc 2% red cell suspension.

Adult guinea pigs of about 500 g weight were used for the experiments. Blood drops for the counts were obtained by a cut through an ear vein. The red cells were counted by the routine method using a Spencer-Neubauer counting chamber and the reti-

culocytes by a dry method using cresyl blue stained cover slips. Red cell agglutination in whole blood was looked for macro- and microscopically in a drop of citrate blood on a glass slide.

The serum injections were given repeatedly intraperitoneally once a day or as a single dose intracardially in ether anesthesia. The hormone preparations used were Cortone (Merck), and Prolongcort (Wilson).<sup>1</sup> The latter is a corticotropin preparation with prolonged action. The hormones were administered intramuscularly twice daily for the first three days and then once daily, if the treatment was continued. When the serum was given intravenously, the hormone treatment was started in the morning of the same day as the immune serum treatment, the first serum injection being given in the afternoon after the second hormone dose. When the serum was given intracardially, the hormone treatment was started the day before. The daily dose of cortisone was 10 mg., and of corticotropin 6 units. The reliability of the eosinophil count of guinea pigs as a dosage guide is not known. However, the effect of the dosage was tested in a separate series of guinea pigs and was found to produce a satisfactory decrease in the eosinophil count. Since many healthy guinea pigs have no eosinophilic leukocytes, those with eosinophils in the blood were selected for the

TABLE 1  
THE EFFECT OF A SINGLE INJECTION OF CORTICOTROPIN OR CORTISONE ON THE EOSINOPHIL COUNT IN GUINEA PIGS

Treatment	Eosinophils per cu mm of Blood	
	Initial Value	After Injection
None .....	44	88
" .....	220	220
" .....	1144	2200
" .....	4477	2700
Prolongcort 3 units .....	44	22
" 3 " .....	440	330
Prolongcort 6 units .....	2200	528
" 6 " .....	2700	176
Cortone 5 mg .....	374	264
" 5 " .....	528	418
Cortone 10 mg .....	88	44
" 10 " .....	198	22

<sup>1</sup> The former was generously supplied by Merck & Co., Inc., and the latter by Wilson & Co., Inc.

test. The eosinophils were counted according to a modification of Randolph's method (17) in the morning, the hormones injected in one dose in the afternoon, and the eosinophils counted again on the following morning. For the effect on the count, see Table 1. The hormone treatment was continued for three days in the first series of animals, for five days in the second, for six days in the third, and for ten days in the last series.

## RESULTS

Four different dosage schedules were used. The animals in the first series (Table 2) were given 8 hemolytic units daily for two

TABLE 2

SERIES 1. EACH ANIMAL WAS GIVEN 8 HEMOLYTIC UNITS PER 100 G BODY WEIGHT INTRAPERITONEALLY ON TWO SUCCESSIVE DAYS. THE FIGURES IN BRACKETS MEAN TIME IN DAYS AFTER THE FIRST SERUM INJECTION

No.	Hormone Treatment	Initial R.B.C.	Lowest R.B.C.	Reticulocyte Peak in %
1	No hormones . . . .	5.43	0.93 (4)	36 (5) Died after 5 days
2	" " . . . .	5.88	1.22 (4)	No response " " 4 "
3	" " . . . .	5.08	1.23 (4)	70.4 (8) " " 8 "
4	Corticotropin . . . .	4.88	0.99 (2)	6.6 (2) " " 2 1/2 "
5	" " . . . .	5.31	4.41 (1)	No response " " 1 1/2 "
6	" " . . . .	4.78	0.79 (3)	15.0 (3) " " 3 "
7	Cortisone . . . . .	5.52	3.12 (2)	No response " " 2 1/2 "
8	" " . . . . .	5.49	1.35 (2)	" " " " 2 1/2 "
9	" " . . . . .	4.88	1.32 (2)	" " " " 2 1/2 "

days. All these animals died, and to our surprise the corticotropin or cortisone treated animals died sooner than the animals given immune serum only. Nothing can be said about the reticulocyte response of the guinea pigs in this series, because the survival time of the animals after the treatment was too short for a reticulocyte peak to appear.

In the second series the animals were treated with smaller doses daily, given intraperitoneally for four days. As seen from Table 3, one of the control animals died of anemia, whereas none of the hormone treated animals died. On the other hand, the red cell count of one of the control animals decreased less than that of the

TABLE 3

SERIES 2. ONE INTRAPERITONEAL INJECTION OF IMMUNE SERUM DAILY FOR FOUR DAYS: THE FIRST TWO DAYS 4 HEMOLYTIC UNITS PER 100 G OF BODY WEIGHT, THE LAST TWO DAYS 2 2/3 UNITS

No.	Hormone Treatment	Initial R.B.C.	Lowest R.B.C.	Reticulocyte Peak in %
10	No hormones ....	4.75	0.95 (4)	34.4 (4) Died after 4 days
11	" " ....	4.53	3.63 (6)	20.8 (6) Recovered
12	" " ....	4.88	1.95 (4)	70.2 (6) "
13	Corticotropin ....	4.36	1.61 (6)	72 (8) "
14	" " ....	5.41	1.63 (6)	61.4 (6) "
15	" " ....	4.90	1.55 (6)	50.6 (8) "
16	Cortisone .....	5.25	1.29 (6)	35.6 (8) "
17	" " .....	5.06	1.37 (6)	78.4 (8) "
18	" " .....	5.15	1.34 (6)	74.5 (8) "

hormone treated animals. Consequently, no distinct effect of corticotropin or cortisone on the hemolysis was seen in this experimental series. The reticulocyte response was slight in guinea pig No. 11, because only mild anemia developed. Guinea pig No. 10 died before the optimal time for a reticulocyte peak. All the other animals, except No. 16, showed about the same degree of reticulocytosis, and no effect of the hormone treatment was observed.

In the third experimental series, the animals were given a single intracardial injection of 8 hemolytic units per 100 g. Marked anemia developed in all these animals, but they recovered. As seen from Table 4, the red cell count decreased more, however, in the hormone treated animals than in the controls, and more in the corticotropin treated guinea pigs than in those given cortisone.

TABLE 4

SERIES 3. THE ANIMALS WERE GIVEN 8 HEMOLYTIC UNITS OF IMMUNE SERUM PER 100 G IN ONE DOSE INTRACARDIALLY

No.	Hormone Treatment	Initial R.B.C.	Lowest R.B.C.	Decrease in R.B.C.	Reticulocyte Peak in %
19	No hormones ....	4.43	1.76 (4)	2.77	38.6 (5) Recovered
20	" " ....	4.45	2.35 (4)	2.10	62 (5) "
21	Corticotropin ....	5.44	1.16 (4)	4.28	45 (7) "
22	" " ....	5.87	1.23 (4)	4.64	45 (7) "
23	Cortisone .....	4.95	1.79 (3)	3.16	60.2 (5) "
24	" " .....	5.38	1.64 (5)	3.74	74 (7) "

The reticulocytosis was slightly higher in the cortisone treated animals than in the other two groups.

The animals of the last experimental series were given a small daily dose of only one hemolytic unit (per 100 g) intraperitoneally on eight successive days. All these animals showed a decrease in the red cell count, but severe anemia did not develop, and all of them recovered. After the last injection the count dropped for two days and then started to rise, except in No. 30 which had the lowest count as early as a day after the last serum injection. As seen from Table 5, two of the control animals showed a bigger

TABLE 5

SERIES 4. THE ANIMALS WERE GIVEN DAILY DOSES OF ONE HEMOLYTIC UNIT OF IMMUNE SERUM PER 100 G INTRAPERITONEALLY ON EIGHT SUCCESSIVE DAYS. ALL THE ANIMALS RECOVERED

No.	Hormone Treatment	R.B.C. before Treatment	Lowest R.B.C. after Treatment	Decrease in R.B.C.	Reticulocytosis	
					6 Days after First Injection	8 Days after First Injection
25	None .....	5.92	2.51	3.41	4.8	20.1
26	" .....	5.68	2.50	3.18	5.4	17.2
27	" .....	5.19	4.01	1.18	3.4	8.8
28	Corticotropin ..	5.54	4.58	0.96	20.0	14.4
29	" ....	5.79	2.68	3.11	16.0	38.2
30	" ....	4.56	3.15	1.41	17.0	29.6
31	Cortisone .....	5.35	3.55	1.80	28.0	23.6
32	" .....	6.12	3.85	2.27	20.8	14.0
33	" .....	5.50	3.96	1.54	13.0	8.0

drop in the red cell count than any of the hormone treated animals. The third control animal, on the other hand, showed only a slight drop in the red cell count. The mean decrease in R.B.C. of the three controls was 2.59 mill., in the corticotropin treated animals 1.82, and in the cortisone treated animals 1.87. Hence it is possible that the hormones counteracted the hemolysis in this series, but the differences in R.B.C. changes are not statistically significant.

All the guinea pigs in the experimental series 1, 2 and 3 developed agglutination of red cells in citrate blood for one to five days after the last serum injection. The method for observing the phenomenon was crude, and no difference in the degree of agglutination

was noticed between the hormone treated animals and the controls. No agglutination was seen in blood taken from animals treated with eight small doses of immune serum (series 4).

#### COMMENT

In the experiments now reported it was obvious that both corticotropin and cortisone enhanced the hemolytic effect of immune serum given in one or two large doses, but the hormones had no effect on the hemolysis if the immune serum was given in four smaller doses. When guinea pigs were treated over a period of eight days with a small intraperitoneal dose of immune serum given daily, there was no statistically significant effect of the hormone treatment on the degree of red cell destruction, in spite of the observation of the largest fall in the red cell count in two of the three control animals suggesting a possible slight protecting effect against hemolysis. In the same series, it was quite obvious that reticulocytosis developed sooner if the animals were treated with corticotropin or cortisone. This suggest an accelerating effect of adrenal steroids on the reticulocyte response to a mild hemolysis, even if the height of the reticulocyte peak is not affected.

On the basis of the results of these experiments it is concluded that the degree of hemolysis, induced by the hemolytic immune serum injected into the animals, may be influenced by simultaneous treatment with corticotropin or cortisone, under certain circumstances. If the hemolytic immune serum is given in high doses, the simultaneous treatment with corticotropin or cortisone seems to enhance the hemolysis, but if the serum is given in moderate doses, the hormones have no effect. Palmer et al. (27) noted a protecting effect of adrenal steroids against hemolysis if the immune serum was given in repeated small doses. The results were similar in our series, but the differences were too small to be statistically significant. It is also possible that the differences between the decrease in R.B.C. in hormone treated animals and controls are simply due to increased red cell production in the hormone treated guinea pigs which responded sooner with reticulocytosis than the others.

As mentioned in the review of the literature, a large number of works have been reported on the influence of corticotropin and cortisone on the different phases of antigen-antibody reactions.

The best explanation of the contradictory results is the assumption that the hormones may influence the process in different directions depending on the doses of antigen, antibody, and hormones used in animal experiments, and probably also on the species of animal. Therefore, it is impossible to draw any definite conclusions on the basis of animal experiments regarding the mode of action of corticotropin and cortisone treatment in acquired hemolytic anemia in man. The results obtained by Palmer et al. (27) with small antibody doses, which are to some extent confirmed by us, show that, under certain circumstances, corticotropin and cortisone may have a slight protecting effect against hemolysis. In addition our experiments show distinctly that the adrenal steroids may accelerate the development of reticulocytosis caused by mild hemolysis *in vivo*, and thus protect the organism by increased red cell production. It seems more likely, however, that the good therapeutic effect of the adrenal steroids in acquired hemolytic anemia in man is mostly due to suppression of antibody formation.

#### SUMMARY

Thirty-three guinea pigs were given injections of hemolytic immune serum in high, moderate, or small doses intraperitoneally, or as a single dose intracardially. One-third of the animals were simultaneously treated with corticotropin and one-third with cortisone. If the serum was given intraperitoneally, in repeated moderate doses, no effect of the hormones on the degree of hemolysis was seen in the experiments. If the animals were given repeated small doses of immune serum, the red cell count decreased somewhat less in the hormone treated animals than in the controls, but the difference was not statistically significant. When the animals were given two large intraperitoneal doses or one large intracardial dose of immune serum, the hormones aggravated the hemolytic anemia induced in the guinea pigs.

In the experiments with repeated small intraperitoneal doses of immune serum, the reticulocyte peak appeared sooner in the hormone treated animals than in the controls, even if the height of the peak was not influenced. In the experiments with higher doses of immune serum there was no marked effect of the hormones on the reticulocyte response to hemolysis.

## REFERENCES

1. BAUMGARTNER, W.: *Helv. med. acta* 1947:14:502.
2. BEST, W. R., LIMARZI, L. R., and PONCHER, H. G.: *J. A. M. A.* 1951: 147:827.
3. CHASE, J. H., WHITE, A., and DOUGHERTY, T. F.: *J. Immunol.* 1946: 52:101.
4. CLEARINKIN, K. P.: *Lancet* I: p. 183, 1952.
5. DAMESHEK, W., ROSENTHAL, M. C., and SCHWARTZ, L. J.: *New Engl. J. Med.* 1951:244:117.
6. DOUGHERTY, T. F., and WHITE, A.: *Endocrinology* 35: 1, 1944.
7. DOUGHERTY, T. F., WHITE, A., and CHASE, J. H.: *Proc. Soc. Exp. Biol. Med.* 1944:56:28.
8. DOWS, P. B.: *J. Pharm. Exp. Therap.* 1951:103:341.
9. EISEN, H. N., MAYER, M. M., MOORE, D. M., TARR, R. R., and STOERK, H. C.: *Proc. Soc. Exp. Biol. Med.* 1947:65:301.
10. FAGRAEUS, A.: *Acta med. scand.* 1948:130:suppl. 204.
11. GARDNER, F. H., Mc ELFRESH, A. E., and HARRIS, J. W.: *J. Lab. Clin. Med.* 1951:37:444.
12. GERMUTH, F. G., and OTTINGER, B.: *Proc. Soc. Exp. Med. Surg.* 1949:74:259.
13. HAM, T. H., and CASTLE, W. B.: *Tr. A. Am. Phys.* 1940:55:127.
14. HAMMOND, C., and NOVAK, M.: *Proc. Soc. Exp. Biol. Med.* 1950:74:155.
15. HARRIS, T. N., GRIMM, E., MARTENS, E., and SCHRICK, W. E.: *J. Exp. Med.* 1945:81:73.
16. HAVENS, W. P., SHAFFER, J. M., and HOPKE, C. J.: *J. Immunol.* 1952:68:389.
17. HENNEMAN, PH. H., WEXLER, H., and WESTENHAVER, M. M.: *J. Lab. Clin. Med.* 1949:34:1017.
18. HERBERT, P. H., and DE VRIES, J. A.: *Endocrinology* 1949:44:259.
19. KASS, E. H., and FINLAND, M.: *New Engl. J. Med.* 1951:244:464.
20. LARSON, D. L., and TOMLINSON, L. J.: *J. Clin. Invest.* 1951:30:1451.
21. LETMAN, H.: *Acta med. scand.* 1953:146:436.
22. MEGEL, H., and GORDON, A. S.: *Endocrinology* 1951:48:391.
23. MENKIN, V.: *Am. J. Physiol.* 1940:129:691.
24. MOESCHLIN, S., BÁGUENA, R., and BÁGUENA, J.: *Int. Arch. Allergy Appl. Immunol.* 1953:4:83.
25. MOESCHLIN, S., PELAEZ, J. R., HUGENTOBLE, F., BÁGUENA, R., BÁGUENA, J., and DEMIRAL, B.: *Comptes rendus du troisième congrès de la Société Internationale Européenne d'Hématologie*, 1951:192.
26. MOESCHLIN, S., ZURUKZOGU, W., and CASTLE, J.: *Acta haemat.* 1953:9:277.
27. PALMER, J. G., CARTWRIGHT, G. E., and WINTROBE, M. M.: *Proceedings of the Second Clinical ACTH Conference*, I: p. 438, Philadelphia 1951.
28. REISS, M., and GÖTHE, J.: *Endokrinologie* 1937:19:148.

29. STOERK, H. C., and STOROVSKY, M.: *Am. J. Path.* 1950:26:708.
  30. THATCHER, J. S., HOUGHTON, B. C., and ZIEGLER, C. H.: *Endocrinology* 1948:43:440.
  31. THORN, G. W., FORSHAM, P. H., FRAWLEY, T. F., HILL, S. R., ROCHE, M., STAECHELIN, D., and WILSON, D. L.: *New Engl. J. Med.* 1950:242:783, 824 and 865.
  32. DE VRIES, J. A.: *J. Immunol.* 1950:65:1.
  33. WASASTJERNA, C.: *Acta med. scand.* 1951:140:suppl. 258.
  34. WELCH, C. S., and DAMESHEK, W.: *New Engl. J. Med.* 1950:242:601.
  35. WINTER, C. A., SILBER, R. H., and STOERK, H. C.: *Endocrinology* 1950:47:60.
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## BIOLOGICALLY ACTIVE PROTEINS FROM WHEAT GERMS

### I

#### AN ANTI-EOSINOPHILIC FRACTION (AEF)

by

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Investigations into the influence of various vegetable proteins on the organism have been chiefly concerned with their toxic property and their ability to agglutinate red blood cells (1, 2, 3). The best known hemagglutinative vegetable proteins are ricin (4), soyin (5) and concavallin A (6). With the exception of the soya bean the plants from which the above hemagglutinative proteins are isolated are of little practical value in human economy. It is noteworthy that biologically active substances which are, or are associated with, proteins, have been isolated from wheat, a common food. Campbell et al. (7) have isolated from wheat flour treated with nitrogen trichloride a toxin which orally administered induces »running feet» in test animals. Andersson et al. (8) have found that wheat proteins have an unfavourable effect on celiac patients, resulting in an aggravation of the symptoms and an impaired general condition.

The study of the proteins of the whole grain is an exceedingly laborious process because the gluten is poorly soluble and difficult to treat. The wheat germ is a considerably easier proposition as it contains relatively little gluten and starch but comparatively large quantities of albumins and globulins. Active substances like vitamin E (9) and sitolipin (10) have been isolated from wheat germ earlier.

The present paper describes a protein fraction with a relatively strong reducing effect on the number of eosinophilic cells of peripheral blood both in human subjects and in test animals. We call this fraction the antieosinophilic fraction (AEF).

#### METHOD OF ISOLATION

10 kg of wheat germs were extracted with 40 litres of 75 per cent acetone containing 1000 ml of concentrated hydrochloric acid, for 16 hours under continued agitation. It was allowed to stand for 1 hour. The supernatant was siphoned off from the germ sediment. The germs were washed once with 70 per cent acetone (5 liters) in a large Büchner funnel. The supernatants were combined, 4 grams of LiCl per liter was added, the pH raised to 5.5 with 2 N LiOH, and the mixture allowed to stand at  $-5^{\circ}\text{C}$  overnight. The precipitate was discarded and the solution poured into 180 liters of acetone at  $-5^{\circ}\text{C}$  under constant stirring and left to stand overnight. The supernatant was siphoned off. After centrifugation the precipitate was dried in a current of air ( $-5^{\circ}\text{C}$ ) until free from acetone. — The powder obtained was suspended in 5 liters of M/10  $\text{Na}_2\text{HPO}_4$  solution, centrifuged in a Sharples supercentrifuge, reextracted with 1 liter of the above phosphate solution, and centrifuged; the supernatants were combined. The precipitate was discarded. Solid ammonium sulphate was added to the solution until half-saturated and the solution was mixed and left to stand overnight. The precipitate was separated by centrifugation, dissolved in 200 ml of water and dialyzed against tap water until free from ammonium sulphate ( $+5^{\circ}\text{C}$ ). The resulting precipitate was separated by filtration. The pH of the solution was brought to 4.5, an equal volume of saturated NaCl solution was added and the solution was allowed to stand overnight at  $+2^{\circ}\text{C}$ . The precipitate was dissolved in 100 ml of M/15 Sørensen phosphate buffer, pH 7.5. The undissolved portion was centrifuged off and the solution was saturated to 0.20 with a saturated ammonium sulphate solution (11). After standing for 2 hours at  $+2^{\circ}\text{C}$  it was centrifuged and the precipitate was discarded. The solution was saturated to 0.25 with ammonium sulphate, allowed to stand for 2 hours, centrifuged and the precipitate discarded. The precipitate of the solution of 0.35 saturation was saved and the supernatant discarded. The precipi-

tate was suspended in 100 ml of water and dialyzed against tap water until free from ammonium salts. The clear filtered solution was fractionated with ammonium sulphate as above. The precipitate obtained with 0.35 saturation was dissolved in distilled water and dialyzed against distilled water at  $+2^{\circ}\text{C}$  until free from salts. Finally the solution (25 ml) with a protein content approx. ten mg per ml was transferred into sterile ampoules and lyophilized.

The work was done throughout at temperatures under  $+5^{\circ}\text{C}$ , except for the dialyzes with tap water, which had a temperature of  $+5^{\circ}$ — $+7^{\circ}\text{C}$ .

AEF dissolves in lyophilized state readily in water, yielding a nearly colorless solution.

#### EXPERIMENTS

An intraperitoneal injection of 10 mg of AEF into a mouse produced no discernible toxic symptoms in the test animal. The injection of varying amounts of AEF into a cat under chloralose anesthesia produced no changes in the blood pressure or the respiration and pulse rates. No changes were observable in the contractions of isolated guinea-pig intestine reacting to histamine. Investigations carried out with the isolated auricle of a rabbit heart revealed no stimulating action of AEF, but in large doses it produced a depressing effect.<sup>1</sup>

In hypophysectomized rats a very slight decrease was seen in the ascorbic acid content in the adrenal, only equal to approx. one-twentieth of the decrease induced by the corresponding amount of ACTH.<sup>1</sup>

AEF has been found to possess a distinct decreasing effect on the number of eosinophils in the peripheral blood. Corresponding experiments carried out with the fraction obtained from a solution of 0.25 saturation with ammonium sulphate failed to produce a similar decrease in the eosinophils.

The experimental animals employed were white rats, into which 0.1 ml of solution containing 10 mg of AEF per ml was injected intraperitoneally. The total leucocyte and the eosinophil counts

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<sup>1</sup> We wish to express our gratitude to Prof. Eeva Jalavisto, Institute of Physiology, University of Helsinki, and to Dr. Christian Hamburger, State Serum Institute, Copenhagen, for their willing assistance.

per cu mm were made before the injection (12) and repeated 4 hours after the injection.

TABLE 1

Rat No.	Before Injection		4 hr. after Injection		Decrease in Number of Eosinophils	
	Leucocytes	Eosinophils	Leucocytes	Eosinophils	Total	Per Cent
1	27,300	827	27,200	213	614	74
2	23,100	583	32,300	133	450	77
3	14,000	213	20,100	133	80	38
4	14,700	760	25,600	186	574	67

As can be seen from Table 1, a considerable numerical reduction occurred in the eosinophils in each case. In rats No. 3 and No. 4 the total leucocytes increased considerably but nevertheless the number of eosinophilic cells decreased in No. 4 by 67 per cent and in No. 3 somewhat less, though as much as 38 per cent. All the test animals proved completely healthy after the experiment.

A comparison of the percentages of eosinophilic cells in the total number of leucocytes (Table 2) shows a considerable decrease, i.e., over 60 per cent, in each of the four cases, the smallest decrease being 63 per cent and the highest 84 per cent.

TABLE 2

Rat No.	Percentages of Eosinophils in the Total Leucocyte Count		Percentage of Decrease
	Before Injection	After Injection	
1	3.0	0.8	73
2	2.5	0.4	84
3	1.6	0.6	63
4	3.8	0.8	79

For corresponding experiments with human subjects we had 6 volunteers at our disposal. Five mg of AEF was administered in a single intramuscular injection of 0.5 ml of a solution containing 10 mg per ml. As with rats, the total leucocyte and eosinophil counts in 1 cu mm were made before the injection and 4 hours after the injection.

TABLE 3

Test Subject No.	Before Injection		After Injection		Decrease in Eosinophils	
	Total Leucocyt.	Eosinoph.	Total Leucocyt.	Eosinoph.	Number	%
1	8,600	178	8,300	56	122	68
1	6,800	111	6,900	56	55	50
1	8,600	281	13,600	67	214	77
2	7,200	156	8,300	100	56	36
2	4,300	89	7,100	33	56	63
3	5,700	922	11,600	778	144	16
4	5,500	78	8,800	44	34	44
5	4,000	156	7,200	67	89	57
6	4,800	67	6,200	22	45	67

Table 3 shows a relatively marked decrease in the number of eosinophils in all cases. The average percentage of decrease was 53 per cent; at its highest it was 77 per cent and at its lowest 16 per cent. (case No. 3). This test subject who had an exceedingly severe neurodermatitis and a relatively high eosinophil count, reacted to the injection with a 100 per cent rise in the total leucocytes, which apparently caused the little decrease in the percentage of eosinophilic cells.

A study of the decrease in the percentage of eosinophils in the total leucocytes (Table 4) shows that it was not under 47 per cent in a single case, the highest value being 85 per cent, the lowest 47 per cent. The mean of the percentages of the total series is 67 per cent.

TABLE 4

Test Subject No.	Percentage of Eosinophilic Cells		Percentage of Decrease
	Before Injection	After Injection	
1	2.1	0.7	67
1	1.6	0.8	50
1	3.4	0.5	85
2	2.2	1.2	47
2	2.0	0.5	75
3	16.2	6.7	60
4	1.4	0.5	64
5	3.9	0.9	77
6	1.4	0.4	71

With two test subjects (No. 1 and 2) the test was repeated using batches prepared at different times. The results varied to some extent but were definitely on the same lines.

#### SUMMARY

A protein fraction (AEF) with a relatively strong antieosinophilic property was isolated from wheat germs.

Intraperitoneal injection of 1 mg of AEF into rats induced a marked reduction in the number of eosinophilic cells in the peripheral blood. The intramuscular injection of 5 mg of AEF into man induced a definitive decrease in the eosinophilic cell count in the peripheral blood.

AEF in the doses employed did not prove toxic.

#### LITERATURE

1. BOYD, W. C., and REGUERRA, M. J.: *Immunol.* 1949:62:333.
  2. RENKONEN, K. O.: *Ann. med. exper. et biol. Fenniae* 1950:28:45.
  3. KOULUMIES, R.: *Ann. med. exper. et biol. Fenniae* 1950:28:160.
  4. KABAT, E. A., HEIDELBERG, M. and BEZER, A. E.: *J. Biol. Chem.* 1948:168:629.
  5. LIENER, I. E., and ROSE, I. E.: *Proc. Soc. Exp. Biol. Med.* 1953:83:539.
  6. SUMNER, J. B., and HOWELL, S. F.: *J. Bacteriol.* 1936:32:227.
  7. CAMPBELL, P. N., WORK, T. S., and MELLANBY, E.: *Biochem. J.* 1951:48:106.
  8. ANDERSSON, C. M., FRAZER, A. C., FRENCH, J. M., GERRARD, J. W., SAMMONS, H. G., and SMELLIE, J. M.: *The Lancet* 1952:CCLXII:836.
  9. EVANS, H. M., EMERSON, O. H., and EMERSON, G. A.: *J. Biol. Chem.* 1936:113:319.
  10. UROMA, E., and LOUHIVUORI, A.: *Ann. med. exper. et biol. Fenniae* 1951:29:227.
  11. TAYLOR, J. F.: *The Proteins*, Vol. I, Part A, p. 54. Academic Press Inc., New York 1953.
  12. THORN, G. W., FORSHAM, P. H., PRURITY, F. T. G., and HILLS, A. G.: *J. Amer. Med. Ass.* 1948:137:1005.
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## EFFECT OF REPEATED EPINEPHRINE AND HISTAMINE INJECTIONS ON EPINEPHRINE MYDRIASIS

by

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(Received for publication Dec. 11, 1953)

In an earlier study (4) we demonstrated increased tolerance to epinephrine in white mice after repeated epinephrine injections in increasing doses. This raised the lethal dose to 2—3-fold, in part of the animals examined at any rate. It was not possible, however, to elucidate fully the reasons underlying this increase. It was thought to be advisable, therefore, to extend the study to cover other reactions caused by epinephrine in the organism, in order to find out whether they were responsible for alterations of the same type.

One of the conditions studied for this purpose was epinephrine-produced mydriasis and its dependence upon treatment with epinephrine. The severity of this condition has been found to be directly proportionate to the strength of epinephrine effect. This observation has, among other things, provided a basis for the biological determination of epinephrine. In the course of the studies reported in the present paper attention was also paid to the mobilisation of epinephrine in the organism found to take place after histamine injection (1, 3, 9). If the injection of histamine is repeated on several successive days in the form of histamine desensibilisation (6), repeated mobilisation of epinephrine takes place in the organism.

### METHODS

The experiments were made on male white mice weighing about 22 g and accustomed to experimentation. They were kept all the time at room temperature (18—20° C) and fed on wheat bread,

oats, and water. They received milk three times a week. The drugs used were epinephrine hydrochloride («Adrenal», Orion), and histamine dihydrochloride (F. Hoffmann — La Roche). Both were diluted with saline and injected subcutaneously. In the present report, the histamine amount used is calculated as histamine base and the epinephrine as epinephrine hydrochloride. Mydriasis was evoked by injecting three doses of epinephrine, viz. 0.75, 1, and 1.5  $\gamma$ /g. The measurement of the eye was carried out by Pulewka's method (13, 14) in a half-darkened room, always at the same time of the day. The microscope used gave a magnification of 16 times. The diameter of the pupil was measured by means of the ocular scale and is given as measure units (1 unit = 0.05 mm). The injections were made subcutaneously with a very fine needle. Before injection the size of the pupil was measured at intervals of 10–15 minutes until no alterations were visible. For the sake of clarity, only the maximum pupil diameter was taken into consideration.

When the role of methodic errors was tested, it was found that the application of the needle of the syringe enlarged the pupil by 3.8 per cent. Injection of saline produced a dilatation of 6.9 per cent. These two values were of a different magnitude than those obtained in the actual tests.

## RESULTS

*Effect of Epinephrine Injections on Pupil Reaction.* — Treatment with epinephrine was effected by injecting, once a day, increasing doses according to the following scheme: (2—4—)6—10—15—20  $\gamma$ /g. Each dose was given three times in succession. After this series of injections, the average size of the pupil was found to have increased a little from the initial value (table 1). In the table, the values

TABLE 1  
SIZE OF THE PUPIL BEFORE AND AFTER EPINEPHRINE TREATMENT (WITH EXTREME VALUES IN BRACKETS)

Before Epinephrine Injection	After Epinephrine Injection	Alteration %
0.61 (0.3—1.1)	0.69 (0.5—1.1)	+13.1 (0—66.7)

TABLE 2

EXTENT OF EPINEPHRINE MYDRIASIS BEFORE AND AFTER EPINEPHRINE TREATMENT (WITH EXTREME VALUES IN BRACKETS)

Epinephrine Dose γ/g	Dilatation of the Pupil, % of the Initial Value		
	Before Treatment	After Treatment	Difference
0.75	+14.7 (0—50.0)	+57.8 (14.3—85.7)	+43.1 (0—69.0)
1.0	+44.5 (0—150.0)	+93.1 (25.0—266.7)	+48.1 (17.2—188.9)
1.5	+82.2 (50.0—166.7)	+110.4 (57.1—200.0)	+18.2 (2.9—70.0)

corresponding to each dose of epinephrine are mean values obtained with ten animals. The increase from the initial value is given per cent in the column marked «alteration». The extent of epinephrine mydriasis before and after treatment is given in table 2. It seems, from the results of the study, that the sensitivity of the pupil to epinephrine is greater after desensibilisation. The difference was particularly clear when small doses were used. When larger doses of epinephrine were used, the difference was in a great measure obscured by the proximity of the initial value to maximal dilatation.

*Sensitivity of Epinephrine Mydriasis Compared to the Body Weight of Normal Mice.* — In order to find out how far the age variations in the balance of the vegetative nervous system affected the severity of mydriasis, experiments were carried out on animals of differing weights. Table 3 records the proportion between the weight of the animal and the degree of pupil dilatation produced

TABLE 3

EXTENT OF EPINEPHRINE MYDRIASIS IN DIFFERENT WEIGHT GROUPS

Epinephrine Dose γ/g	Dilatation of the Pupil in the Different Groups, % of the Initial Value		
	Under 20 g	20—25 g	Over 25 g
0.75	+18.0	+15.8	+12.9
1.0	+46.7	+30.2	+49.4
1.5	+70.7	+58.2	+65.6

TABLE 4

SIZE OF THE PUPIL BEFORE AND AFTER HISTAMINE DESENSIBILISATION (WITH EXTREME VALUES IN BRACKETS)

Before Desensibilisation	After Desensibilisation	Alteration %
0.50 (0.3—0.7)	0.68 (0.5—1.2)	+36.0 (0—113.4)

by epinephrine. The series consisted of more than 200 mice. No clear-cut differences were noted between the various weight groups, which suggests that the weight and, accordingly, age of the animal do not affect the extent of the mydriasis.

*Histamine Desensibilisation and Epinephrine-produced Mydriasis.*

— Histamine desensibilisation was carried out by the method proposed by Fabinyi and Szebehely (5). The mice were given histamine subcutaneously, on the first three days 2 mg once daily, on the following three days 3 mg. After this, 2 mg was given twice daily for three days, and eventually 3 mg twice daily for the last three days. After this procedure, there was no fall of 3—4° C in body temperature, noted in normal mice after the subcutaneous injection of 100  $\gamma$ /g histamine. This series consisted of 70 mice. In 30 cases the extent of epinephrine mydriasis was determined prior to desensibilisation, and this was repeated one day after the last injection of histamine. The size of the pupil before and after histamine desensibilisation is seen from table 4. This table shows that

TABLE 5

EXTENT OF EPINEPHRINE MYDRIASIS BEFORE AND AFTER HISTAMINE DESENSIBILISATION (WITH EXTREME VALUES IN BRACKETS)

Epinephrine Dose $\gamma$ /g	Dilatation of the Pupil, % of the Initial Value		
	Before Desensibilisation	After Desensibilisation	Difference
0.75	+17.4 (0—50.0)	+68.6 (37.5—113.3)	+51.2 (22.8—83.3)
1.0	+40.6 (25.0—125.0)	+74.1 (33.4—120.0)	+33.5 (16.7—60.0)
1.5	+88.0 (50.0—140.0)	+102.4 (66.7—140.0)	+14.4 (40.0—70.0)

TABLE 6

LETHAL DOSE OF EPINEPHRINE AFTER HISTAMINE DESENSIBILISATION. BRACKETS: PERCENTILE MORTALITY OF NORMAL MICE IN A SIMULTANEOUS TEST (ECKERT, PAASONEN, AND PELTOLA 1951)

Epinephrine Dose $\gamma/g$	No. of Mice	Deaths	Mortality Rate %
4	10	2	20 (12.8)
6	20	6	30 (45.4)
10	20	16	80 (82.7)
20	4	4	100 (100)

permanent dilatation of the pupil was more distinct after histamine than after epinephrine desensibilisation. After histamine desensibilisation the pupil was also found to be more sensitive to epinephrine (table 5); in this case, too, the differences were more distinct when small doses of epinephrine were used.

*The Effect of Histamine Desensibilisation on the Lethal Dose of Epinephrine.* — In order to find out whether the histamine desensibilisation was accompanied by an increase of epinephrine resistance, desensibilised mice were given epinephrine subcutaneously in varying doses. Table 6 records the results of these tests. The percentages given in brackets show the results obtained simultaneously with similar normal mice, taken from a previous report (4). There was no clear-cut difference between the two groups, which suggests that histamine desensibilisation is not accompanied by any increase in resistance to epinephrine.

#### DISCUSSION

Repeated injection of epinephrine in increasing doses seems to augment in a mouse the mydriasis caused by epinephrine, particularly when small doses of this drug are used. The result is in seeming disagreement with earlier observations, according to which resistance to epinephrine increases under similar conditions. (4). On the other hand, however, the response of the pupil to adrenergic stimuli is known to differ from that of the other parts of the body in many respects. Epinephrine mydriasis is not inhibited by ergotamine and is inhibited only in a limited degree by other potent adrenolytic

drugs (11). In some experiments (12), for example, antihistamine phenindamine increased the effect of epinephrine on the iris, while in other tests it was sympatholytic. It is worthy of note, too, that Kato and Watanabe (8), who injected epinephrine subcutaneously into a cat for three weeks, reported the appearance of miosis instead of mydriasis after injecting epinephrine into the carotid artery.

It seems reasonable to assume that, as a result of repeated injections of epinephrine, the equilibrium between the sympathetic and parasympathetic systems is disturbed in one way or another. Such a disturbance of equilibrium may produce rather surprising reactions, such as the sensibilisation of the pupil to epinephrine after the removal of the sympathetic superior cervical ganglion (10). Down to quite recent times the factors underlaying this phenomenon remained unknown. In 1951, however, Robinson (15) found that the iris contains some amino-oxidase which decomposes epinephrine. If the superior cervical ganglion is removed, the amino-oxidase content of the iris falls even to 30 per cent of the normal amount (2). This, of course, increases the effect of epinephrine. Whether, in the experiments now reported, the repeated injections of epinephrine introduced large amounts of this drug into the pupil and thus decreased its amino-oxidase content and increased the mydriasis is not possible to say. The persistent dilatation of the pupil after epinephrine desensibilisation, suggesting the presence of continuous sympathicotonia, spoke for such a possibility.

Although the response of a young animal to epinephrine has been reported to differ from that of an old animal, particularly as far as the cardiovascular system is concerned (7), no such difference was noted in the experiments now reported between mice of different weights and, accordingly, of different ages. It has to be noted, however, that even the smallest mice used by us were adult, though young, individuals.

After histamine desensibilisation, the dilatation of the pupil, when compared with the initial values, was even more marked than after the injection of epinephrine. After histamine injection, the pupil was also more sensitive to epinephrine than before. Both alterations were, thus, similar to those noted after the injection of epinephrine, suggesting the presence of a common pathogenetic mechanism. On the other hand, however, the alteration in the lethal dose of epinephrine was different from that noted after

epinephrine treatment. Concerning this point it should be noted that the epinephrine amounts liberated during histamine desensibilisation are much smaller than those needed for increased epinephrine tolerance.

#### SUMMARY

The object of the study here reported was to find out whether repeated injections of epinephrine produced alterations of epinephrine mydriasis in mice suggestive of an increased resistance to this drug. An attempt was also made to find out how far the repeated epinephrine mobilisation caused by repeated injections of histamine (histamine desensibilisation) gave rise to similar alterations.

The mice received daily increasing amounts of epinephrine in subcutaneous injections, with a final dose of 20  $\gamma$ /g, which is nearly three times greater than LD 50. After the injections the pupil was found to be larger than at the beginning of the test, and the sensitivity of the pupil to epinephrine was likewise increased. The weight of the animal did not play any noticeable part in the intensity of the response. The result was in agreement with earlier observations, according to which the response of the pupil to epinephrine differs from that of the other parts of the body. After histamine desensibilisation the pupil of the mouse was also larger than at the beginning of the test and it was more sensitive to epinephrine, as after epinephrine injections. Yet histamine desensibilisation did not affect the lethal dose of epinephrine.

#### REFERENCES

1. BURN, J. H., and DALE, H. H.: *J. Physiol.* 1926:61:85.
2. BURN, J. H., and ROBINSON, J.: *J. Physiol.* 1952:116:21.
3. DALE, H. H.: *Brit. J. Exp. Med.* 1920:1:103.
4. ECKERT, D., PAASONEN, M., and PELTOLA, P.: *Ann. med. exper. et biol. Fenniae* 1951:29:110.
5. FABINYI, M., and SZEBEHELY, J.: *Acta Allergol.* 1949:2:233.
6. HAAS, H.: *Histamin und Antihistamine II*, Württemberg 1952.
7. HARTMAN, F. A., and KILBORN, L. G.: *Am. J. Physiol.* 1918:45:11.
8. KATO, T., and WATANABE, M.: *Tohoku J. Exper. Med.* 1920:1:73, quoted by HARTMAN, F. A., and BROWNELL, K. A.: *The Adrenal Gland*, Philadelphia 1949.

9. MACKAY, M. E.: *J. Pharmacol. Exper. Therap.* 1929:37:349.
  10. MELTZER, S. J., and AUER, C. M.: *Am. J. Physiol.* 1904:11:28.
  11. NICKERSON, M.: *Pharmacol. Rev.* 1949:1:27.
  12. PAASONEN, M. K.: *Ann. med. exper. et biol. Fenniae* 1953:31:Suppl. 7.
  13. PULEWKA, P.: *Arch. exper. Path. u. Pharmacol.* 1932:168:307.
  14. PULEWKA, P.: *Ibidem* 1939:180:119.
  15. ROBINSON, J.: *J. Physiol.* 1952:116:21.
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## REACTION OF THE THYROID GLAND DURING HISTAMINE SHOCK AND PROLONGED TREATMENT WITH HISTAMINE

by

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The toxin theory of shock has been almost universally accepted. It is based on H. H. Dale's and P. P. Laidlow's (6) hypothesis regarding liberation of histamine and supported by investigations revealing liberation of a substance with the pharmacodynamic effect of histamine, the «H-substance» (10, 11, 7). Moreover, in 1935, after the successful chemical identification of histamine in tissues, the histamine theory has found an increasing number of advocates. Histamine is now frequently used in various tests on animals for study of shock, and, for instance of the action of both acute and delayed shock on different organs.

As to the majority of glands with internal secretory activity, there is but sparse information in the literature on their reaction to shock, especially to shock caused by histamine. Reports are scanty also with regard to the reaction of these organs during prolonged treatment with histamine. This refers especially to the thyroid gland. Since glands with internal secretory activity are of great clinical significance in connection with a variety of shocks, this investigation was made in order to contribute to the knowledge of the effect of histamine on the thyroid gland.

## PREVIOUS INVESTIGATIONS

In the literature, reports on the effect produced on the thyroid by various kinds of stress are to some extent contradictory. This, H. Selye (23, 24) considers due to the type of thyroid reaction, which is generally bi-phasic and highly dependent on conditioning factors. According to S. Dvoskin (9), the formation of intra-epithelial colloid droplets in the thyroid of rats, due to administration of thyrotropin, may as well be caused by several toxic substances. One of these is histamine and, according to him, the stimulating effect is also valid in hypophysectomized animals. A contradictory view is mentioned by A. Costa *et al.* (5). Fixation of radio-iodine in the thyroid shows that the activity of the gland is not affected by histamine. In this connection it should be mentioned that thyroidectomy in rats reduces the histamine content in skin and other tissues, whereas the opposite effect is gained by thyroid medication (12). That hypophysectomy may reduce the resistance to histamine and to anaphylactic shock is a parallel observation in some measure (3, 21, 22, 25, 34).

Tests on rats exposed to tourniquet shock showed a reversible disturbance in absorption of radio-active iodine by the thyroid, and this was interpreted as a sign either of increased or decreased thyroid activity (13).

In recent years, explanations of various phenomena have been sought on the basis of the General-Adaption-Syndrome, and these theories have incited new investigations. Attempts to explain the reaction of various organs according to Selye's theories have been made. It is well known that cortisone reduces the capacity of the thyroid to absorb  $I^{131}$  (4), and both ACTH and cortisone reduce the amount of serum-protein iodine (14). According to Selye's theory, the effect of various kinds of stress on the thyroid is conveyed via the adrenal gland. Unspecific stress in different forms, for instance alloxan, formaldehyde, diphtherotoxin, starvation, cold, and emotional stress, has been used in order to test the reaction of the thyroid (23, 24). The results have often been difficult to interpret, and in some cases contradictory evidence has been found.

## THE PRESENT INVESTIGATION

Sixty-two young male guinea-pigs, as equal in weight as possible, were used for the tests. Some of the guinea-pigs also served for a study of gastric ulcers by one of us (18). The majority of the animals weighed 450 to 550 grammes, a small minority being under or over-weight. The guinea-pig is generally considered most suitable for thyroid studies, as its gland is inactive and sensitive to reactions (1). The guinea-pigs were kept isolated in a room with windows, at a temperature of  $20\text{ C} \pm 2\text{ C}$ . They were fed with standardized food, consisting of hay, fresh grass, oats, and turnips. The tests were made in late spring and summer, and completed in autumn.

Prolonged action of histamine was aimed at by embedding the histamine in a mixture of beeswax and paraffin oil, as suggested by S. H. Walpole *et al.* (32) and by L. J. Hay (15) and his associates. Histamine dihydrochloride (Hoffman-La Roche<sup>1</sup>) was used in these tests. However, in the pre-tests, the semi-solid mass, prepared exactly according to L. J. Hay *et al.*, containing about 100 mg of histamine base per cubic centimetre, was found to be too elastic and extremely difficult to administer in the exact dose required. The mixture was modified, and instead of the prescribed 600 mg of histamine dihydrochloride, 0.8 cc of beeswax and 2.8 cc of mineral oil, the mixture now contained 4.6 cc of mineral oil — other quantities the same as before. The sum total of the inactive components was thus one and a half times larger than that prescribed in the original method [ $0.8 + 4.6 = 1.5 (0.8 + 2.8)$ ]. A slightly more liquid and less elastic mixture was obtained and injection facilitated. Preparation of the mixture is described in detail in another paper by one of us (18). The test animals were given a dose of this mixture, equal to about 2 mg of histamine base, once daily, injected into the buttocks. They tolerated this dose well as a rule, although the lethal dose for guinea-pigs is only 0.3—0.4 mg/kg of body weight, when injected intravenously (8). The controls were simultaneously given an injection of 0.1 ml of saline. The animals were decapitated about 6 hours after the last injection. Those succumbing to acute

<sup>1</sup> Our thanks are due to S. A. F. Hoffman-La Roche & Co. Ltd. Co., for supplying histamine for the tests.

shock were also decapitated at once when it became evident that recovery was impossible.

The soft parts of both thyroid lobes were carefully removed, the lobes were weighed, and generally immersed in fresh Bouin's fluid at once. Fixation took 12 hours as a rule. The preparations were embedded in paraffin and both lobes were first cut longitudinally into 50 per cent sections with a microtome, and about 10 slices of 6–8  $\mu$  were taken from the medial sections. Mallory's azan stain, modified by Koneff, was used. This method was found adequate by P. Tala (26), and by some others, in that well-stained preparations with good contrasts were obtained, as was the case in this investigation, too.

The activity of the thyroid was measured according to the histo-quantitative method worked out by U. Uotila and O. Kannas (30) — on the basis of Uotila's (27, 28, 29) investigations —, and used also by P. Tala.

Student's *t* test was used for statistical assessment of the average difference in the two groups. Determinations were made on the basis of the compiled tables as to the difference, whether almost significant, significant, or highly significant.

#### RESULTS

The tests were made in two series, I and II. The first series included 41 guinea-pigs, *i.e.* animals subjected to prolonged histamine action for 12 or 16 days, and animals succumbing to shock after the first injection of histamine. The second series contained 21 guinea pigs, *i.e.* animals killed after the first or third injection. There were 5 controls in each series.

All the guinea-pigs were given one daily intramuscular injection of about 2 milligrams of histamine base. Some of them succumbed to shock, as a rule 20 to 60 minutes after one of the injections, the majority after the first one. The surviving guinea-pigs regularly showed signs of lassitude, huddling up, with hairs on end. Breathing was laboured and the pulse rate increased. They tried to wipe off something non-existent from their noses with their paws which is a sign of difficulty in breathing. They often jerked their heads, making sounds like vomiting or retching. Some of them lay down flat on their side with clonic spasms of the limbs. And yet they

survived the shock at times. Before dying, all the animals had clonic spasms. Finally they lay perfectly still, panting for breath. Breathing rate was highly retarded.

The results of determining the proportion between the different glandular components: epithelium (E), colloid (C), and stroma (S), are given in tables 1 and 2 containing the results obtained in test series I and II.

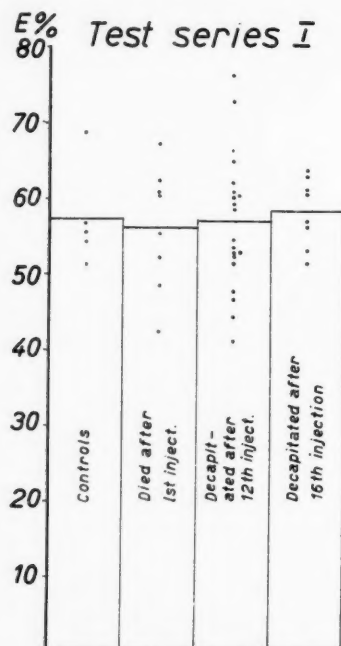


Fig. 1. — Test series I. Each column shows the average percentage of thyroid epithelium in the different test groups.

On close study of the percentages of the different components in Table 1, it is seen that there is *no statistical difference between controls and animals dying after the first injection of histamine, or between the former and those subjected to prolonged histamine treatment for 12 or 16 days*. As a rule the percentage of stroma was not displaced to any extent due to activity experiments of the thyroid (16, 26).

In Fig. 1, the average percentage of epithelium in each group is presented in the form of columns, and observations in the several

cases are given. The divergencies from the »control line» are small in the different test groups.

It may be assumed that the thyroid of the animals dying after the first histamine injection had not had time to undergo alterations denoting changed activity due to histamine, as they died 20 minutes to well over an hour after the injection. Therefore, test series II was started. In it, the guinea-pigs were killed about 6 hours after

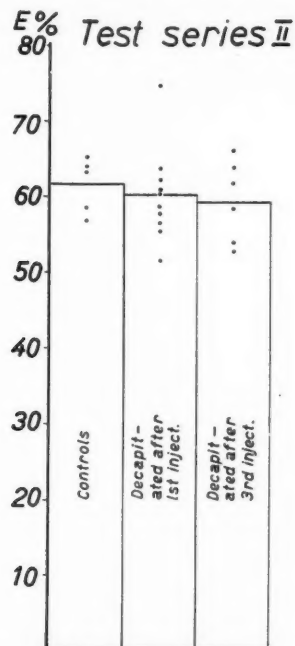


Fig. 2. — Test series II. Each column gives the average percentage of thyroid epithelium in the different test groups.

the first or third injection. — At this point it should be mentioned that P. Tala (26), in his investigations, showed that the activity of the thyroid changes 30 minutes after injection of thyrotropin.

The results obtained in series II (Table 2) prove the correctness of those obtained in series I. *No tenable statistical difference between the average percentages of epithelium and colloid, compared with the controls, occurred.* However, a decrease of  $1.3 \pm 0.6$  per cent in the stroma was noted after three histamine injections. This difference is almost significant.

TABLE 1  
TEST SERIES I  
ANIMALS DYING OF SHOCK AFTER THE FIRST INJECTION OF HISTAMINE IN BEESWAX, AND THOSE DECAPITATED AFTER PROLONGED HISTAMINE TREATMENT FOR 12 AND 16 DAYS. (E — EPIHELIM; C — COLLOID; S — STROMA)

Treatment May 19—Aug. 11 1953	No.	E %	C %	S %	Weight of animal on termi- nating treatment (g)	Weight alteration in weight (g)	Weight of thyroid (gm)	Erosion or ulceration of stomach
1	130	54.2	42.2	3.6	500	+ 75	63	+
Controls	131	51.2	45.2	3.6	500	± 0	61	
	132	68.7	27.1	4.2	470	+ 110	71	
	133	56.8	40.8	2.4	500	+ 95	66	
	134	55.5	41.4	3.1	515	+ 65	61	
Average ± standard dev.		57.3 ± 3.0	39.3 ± 3.2	3.4 ± 0.3		+ 69.0 ± 18.9	64.4 ± 1.9	
2	11	62.4	33.1	4.5	—	—	—	
Died 20 min. to 1 hour after in- jection of about 2 mg. histamine in beeswax	12	60.9	37.5	1.6	—	—	—	
	13	67.2	31.3	1.5	—	—	—	
	92	60.4	35.7	3.9	—	—	61	
	93	48.3	41.4	10.3	—	—	69	
	96	55.3	43.1	1.6	—	—	68	
	98	42.4	55.2	2.4	—	—	78	
	Y.1	52.0	45.1	2.9	—	—	—	
Average ± standard dev.		56.1 ± 2.9	40.3 ± 2.7	3.6 ± 1.0	—		69.0 ± 3.5	
3	33	60.1	37.0	2.9	555	— 65	55	+
Daily injection of about 2 mg. histamine in beeswax for 12 days. Decapita- tion 6 hours after last injection	34	53.5	42.2	4.3	670	— 100	47	+
	35	54.4	43.1	2.5	730	— 35	78	+
	36	64.8	31.0	4.2	515	— 45	71	+
	37	72.9	25.9	1.2	590	— 70	57	+
	38	76.2	21.5	2.3	545	— 10	56	—
	39	59.2	38.9	1.9	570	— 70	64	+
	40	60.8	35.9	3.3	595	— 25	38	+

	41	66.2	31.3	2.5	515	440	— 75	51	+
	42	62.0	33.7	4.3	585	560	— 25	—	+
	89	46.5	50.7	2.8	480	510	+ 30	69	+
	90	51.3	44.0	4.7	435	490	+ 55	66	+
	91	58.5	38.7	2.8	485	520	+ 35	47	—
	94	47.6	49.2	3.2	410	430	+ 20	46	—
	95	60.2	36.0	3.8	440	490	+ 50	55	—
	97	52.8	41.6	5.6	385	420	+ 35	59	—
	100	41.0	55.9	3.1	420	435	+ 15	49	+
	101	52.2	45.6	2.2	400	410	+ 10	44	—
	102	44.3	52.5	3.2	360	375	+ 15	50	—
	113	52.8	43.5	3.7	600	620	+ 20	72	+
Average $\pm$ standard dev.		56.9 $\pm$ 2.0	39.9 $\pm$ 2.0	3.2 $\pm$ 0.2			— 11.8 $\pm$ 10.5	56.5 $\pm$ 2.5	
4 Daily injection of about 2 mg. histamine in beeswax for 16 days. Decapita- tion 6 hours after last injection	25	53.0	44.8	2.2	530	550	+ 20	—	—
	26	56.1	42.7	1.2	570	560	— 10	—	—
	27	61.0	37.9	1.1	530	535	+ 5	—	—
	28	62.9	35.6	1.5	475	490	+ 15	—	+
	29	56.8	40.4	2.8	510	560	+ 50	—	+
	30	51.2	47.1	1.7	530	505	— 25	—	—
	31	63.6	34.6	1.8	450	465	+ 15	—	+
	32	60.5	35.6	3.9	580	580	— 0	—	+
Average $\pm$ standard dev.		58.2 $\pm$ 1.6	39.8 $\pm$ 1.6	2.0 $\pm$ 0.3			+ 8.8 $\pm$ 7.9		
Test I			* E % $\Delta \pm S_d$	C % $\Delta \pm S_d$	S % $\Delta \pm S_d$	Weight of thyroid $\Delta \pm S_d$	Weight of animal $\Delta \pm S_d$		
	1 histamine injection — controls		— 1.2 $\pm$ 4.4	+ 1.0 $\pm$ 4.2	+ 0.2 $\pm$ 1.1	+ 4.6 $\pm$ 3.7	—		
	12 histamine injections — controls		— 0.4 $\pm$ 4.4	+ 0.6 $\pm$ 4.2	— 0.2 $\pm$ 0.5	— 7.9 $\pm$ 5.0	— 80.8 $\pm$ 23.1		
	16 histamine injections — controls		+ 0.9 $\pm$ 3.1	+ 0.5 $\pm$ 3.2	— 1.4 $\pm$ 0.5	—	— 60.2 $\pm$ 17.7		

\*  $\Delta$  = difference in averages.  $S_d$  = deviation of difference in averages.

TABLE 2  
TEST SERIES II

ANIMALS DECAPITATED AFTER 1ST OR 3RD INJECTION OF HISTAMINE IN BEESWAX. (E — EPITHELIUM; C — COLLOID; S — STROMA)

Treatment Nov. 7—9 1953	No.	E %	C %	S %	Weight of animal on starting treatment (g)	Weight on termi- nating treatment (g)	Alteration in weight (g)	Weight of thyroid (gm)
1  Controls	135	56.9	38.3	4.8	580	580	± 0	67
	136	65.2	28.6	6.2	490	480	-10	63
	137	63.4	33.0	3.6	510	510	± 0	80
	138	58.5	37.9	3.6	550	545	-5	68
	138	64.0	32.9	3.1	520	490	-30	40
Average ± standard dev.		61.6 ± 1.6	34.1 ± 1.8	4.3 ± 0.6			-9.0 ± 5.6	63.6 ± 6.5
2  One injection of about 2 mg histamine in beeswax. De- capitation 6 hours after injection	140	56.5	40.3	3.2	530	520	-10	41
	141	62.1	33.6	4.3	520	520	± 0	40
	142	63.8	32.8	3.4	590	580	-10	49
	143	57.7	40.3	2.0	545	545	± 0	42
	144	55.5	41.6	2.9	535	520	-15	56
	145	74.7	21.6	3.7	515	515	± 0	39
	146	60.9	35.1	4.0	480	475	-5	45
	147	58.9	38.0	3.1	470	465	-5	38
	148	60.3	34.5	5.2	580	575	-5	43
	149	51.5	45.9	2.6	500	500	± 0	44
Average ± standard dev.		60.2 ± 2.0	36.4 ± 2.1	3.4 ± 0.3			-5.0 ± 1.7	43.7 ± 1.7

3	150	53.8	43.4	2.8	460	440	-20	42			
Daily injection	151	52.6	44.2	3.2	420	400	-20	42			
of about 2 mg	152	63.7	33.2	3.1	405	385	-20	37			
histamine in	153	61.6	35.2	3.2	480	465	-15	44			
beeswax for 3	154	66.0	31.9	2.1	390	385	- 5	42			
days	155	58.5	38.1	3.4	435	425	-10	50			
Average $\pm$ standard dev.		$59.3 \pm 2.2$	$37.7 \pm 2.1$	$3.0 \pm 0.2$			$-15.0 \pm 2.6$	$42.8 \pm 1.7$			
		E % $\Delta \pm S_d$		C % $\Delta \pm S_d$		S % $\Delta \pm S_d$		Weight of thyroid $\Delta \pm S_d$		Weight of animal $\Delta \pm S_d$	
1 histamine injection	— controls	$-1.4 \pm 3.0$		$+2.3 \pm 3.3$		$-0.9 \pm 0.6$		$-19.9-6.8$		$+4.0 \pm 5.8$	
3 histamine injections	— controls	$-2.3 \pm 2.8$		$+3.6 \pm 2.8$		$-1.3 \pm 0.6$		$-20.8-6.8$		$-6.0 \pm 5.8$	

TABLE 3

ANIMALS GIVEN 12 OR 16 INJECTIONS EACH OF HISTAMINE IN BEESWAX DIVIDED ON THE BASIS OF OCCURRENCE OF EROSIONS OR ULCERATIONS IN THE STOMACH.  
(E — EPITHELIUM; C — COLLOID; S — STROMA)

	No.	Number of inj.	E %	C %	S %
1  Animals with erosions or ulcers	27	16	61.0	37.9	1.1
	28	16	62.9	35.6	1.5
	29	16	56.8	40.4	2.8
	31	16	63.6	34.6	1.8
	32	16	60.5	35.6	3.9
	33	12	60.1	37.0	2.9
	34	12	53.5	42.2	4.3
	35	12	54.4	43.1	2.5
	36	12	64.8	31.0	4.2
	37	12	72.9	25.9	1.2
	39	12	59.2	38.9	1.9
	40	12	60.8	35.9	3.3
	41	12	66.2	31.3	2.5
	42	12	62.0	33.7	4.3
	89	12	46.5	50.7	2.8
	90	12	51.3	44.0	4.7
	100	12	41.0	55.9	3.1
	113	12	52.8	43.5	3.7
Average $\pm$ standard deviation			58.4 $\pm$ 1.8	38.7 $\pm$ 1.7	2.9 $\pm$ 0.3
2  Animals without erosions or ulcers	25	16	53.0	44.8	2.2
	26	16	56.1	42.7	1.2
	30	16	51.2	47.1	1.7
	38	12	76.2	21.5	2.3
	91	12	58.5	38.7	2.8
	94	12	47.6	49.2	3.2
	95	12	60.2	36.0	3.8
	97	12	52.8	41.6	5.6
	101	12	52.2	45.6	2.2
	102	12	44.3	52.5	3.2
Average $\pm$ standard deviation			55.2 $\pm$ 2.8	42.0 $\pm$ 2.0	2.8 $\pm$ 0.4
		E % $\Delta \pm S_d$	C % $\Delta \pm S_d$	S % $\Delta \pm S_d$	
(Ulcers +) — (Ulcers —)		+3.2 $\pm$ 3.1	—3.3 $\pm$ 3.0	+0.1 $\pm$ 0.5	

*It is thus conclusive that no changes in the activity of the thyroid, evaluated on the basis of Uotila's and Kannas' histo-quantitative method, occur after a daily intramuscular injection of histamine in beeswax into guinea-pigs, neither in those dying 20 minutes to well over an hour after the first injection, nor in those killed about 6 hours after the first, third, twelfth, or sixteenth injection.*

The guinea-pigs undergoing prolonged histamine treatment for 12 or 16 days (Series I) often exhibited erosions or ulcers in the stomach. In order to discover whether these circumstances might affect the activity of the thyroid, Table 3 was set up and the two groups were compared with regard to proportion of epithelium, colloid, and stroma in the thyroid. *No statistically reliable difference was noted.*

The weight of the guinea-pigs (Table 1) during prolonged histamine treatment (12 and 16 days), was found to vary, being sometimes much lower, sometimes slightly higher than initially. It is evident that the animals with erosions or ulcers lost more weight than did the others. Comparing the weight of the guinea-pigs given 12 or 16 injections of histamine with the controls, it was observed that the former lost on an average  $80.8 \pm 23.1$  and  $60.2 \pm 17.7$  g during the tests. These differences are statistically significant. The weight of the controls rose by  $69.0 \pm 18.9$  g during the tests.

The weight of the thyroid, compared with that of the controls may fall somewhat during histamine treatment, provided the animals do not die, but are killed 6 hours after the first or third histamine injection (Series II). After the first and third injections, the differences noted were  $19.9 \pm 6.8$  and  $20.8 \pm 6.8$  milligram which is almost significant. On the other hand, there was no difference in the weight of the thyroid compared with the controls if the animals died 20 minutes to well over an hour after the histamine injection, or if they were killed 6 hours after the twelfth injection. — It should be mentioned here that A. N. Kuusisto (16), who demonstrated that the effect of certain digitalis glucosides on the thyroid is distinctly inactivating, found no changes in the weight of the gland.

## DISCUSSION

In these tests there was no evidence of histamine in beeswax, injected once daily, affecting the activity of the thyroid — considering the proportions of the various glandular elements: epithelium, colloid, and stroma. In one of the groups the stroma seemed to be somewhat decreased (after 3 histamine injections), but the proportion of this component is not in general considered liable to change (26) and is therefore frequently ignored (16). This observation, *i.e.* that histamine does not affect the proportions of epithelium and colloid — the compounds of the thyroid generally considered active — agrees completely with an observation made by O. Volterrani and E. Marchis (33) which reached us quite recently, that tests with  $I^{131}$  have revealed retention of radio-active iodine to be normal in histamine shock.

With the toxin theory in mind, it is important to state that, anyhow in the animals studied here, histamine did not seem to affect the activity of the thyroid. Conditions seemed to differ in anaphylactic shock. Costa *et al.* (5) have shown that retention of  $I^{131}$  seemed to be initially inhibited and later reduced, in comparison with the controls. Guinea-pigs were used for the tests. The results of tests with different forms of stress, such as alloxan and formalin (2, 20), point in the same direction, while experiments with formaldehyde (19) revealed no changes in the gland as to retention of  $I^{131}$ . The assumed bi-phasic response of the thyroid is made responsible for the divergence.

## SUMMARY

The effect of histamine on the activity of the thyroid was studied in 62 male guinea pigs, including controls. All the animals were of the same weight as far as possible. Histamine in beeswax was used in order to obtain regular and delayed effects. A daily dose of about 2 milligram histamine base was given. Eight of the guinea-pigs succumbed to shock 20 minutes to well over an hour after the first injection. These, and the test animals, which were decapitated 6 hours after the twelfth or sixteenth injection, a total of 41, including controls, formed series I. Animals killed 6 hours after the first or third injection were comprised in series II.

No effect on the activity of the thyroid was found on examination of the distribution in per cent of the different components of the gland: epithelium, colloid, and stroma, with U. Uotila and O. Kannas' histo-quantitative method. This result is pointed out, bearing in mind the toxin theory of shock and its clinical significance.

With prolonged histamine treatment (12 or 16 injections), the fall in weight of the animal is significant. Also the weight of the thyroid seems to decrease after one or three injections, provided the animals survived; this difference, compared with the controls, is almost significant. No change in weight of the organ occurred in the animals dying of shock 20 minutes to well over an hour after the first injection, or in those decapitated after the twelfth injection.

The diminution in weight after prolonged histamine treatment seemed to be greatest in the animals with erosions or ulcers in the stomach. There was no reliable statistical difference, however, in the distribution in per cent of the components of the thyroid, compared with the animals without these manifestations.

#### REFERENCES

1. ARON, M.: *Compt. rend. Soc. de biol.* 1932:110:716.
2. ARVY, L., and GABE, M.:
3. BANTING, V. G., and GAIRNS, S.: *Am. J. Physiol.* 1926:77:100.
4. BERSON, S. A., and YALOW, R. S.: *J. Clin. Endocrinol.* 1952:12:407.
5. COSTA, A., VOLTERRANI, O., MARCHIS, E., BENEDETTO, V., and SCORTA, A.: *Boll. Soc. ital. biol. sper.* 1952:28:557, as referred to by *Excerpta Medica Sectio 3*, 1953:7:325.
6. DALE, H. H., and LAIDLAW, P. P.: *J. Physiol.* 1910:41:318.
7. DRAGSTEDT, C. A., and GEBAUER-FUELNEGG, E.: *Am. J. Physiol.* 1932:102:520.
8. DOERR, R.: *Die Immunitätsforschung. Die Anaphylaxie II, Immunitätsreaktion und endogene Vergiftung.* Springer-Verlag, Wien 1951.
9. DWOSKIN, S.: *Endocrinology* 1948:43:52.
10. FELDBERG, W., and NAGEL, E.: *Arch. f. d. ges. Physiol.* 1932:230:129.
11. FELDBERG, W., and NAGEL, E.: *Arch. f. d. ges. Physiol.* 1932:230:674.
12. GOTEL, F. R., and DRAGSTEDT, C. A.: *Proc. Soc. Exper. Biol. & Med.* 1940:45:688.
13. HAMOLSKY, M. W., GIERLACH, Z. S., and JENSEN, H.: *Federation Proc.* 1950:9:181, as referred to by SELYE.

14. HARDY, J. D., RIEGEL, C., and ERISMAN, E. P.: *Am. J. M. Sc.* 1950: 220:290.
  15. HAY, L. J., VARCO, R. L., CODE, CH. F., and WANGENSTEEN, O. H.: *Surg. Gynec. Obst.* 1942:75:170.
  16. KUUSISTO, A. N.: *Acad. Diss., Ann. med. exper. biol. Fenniae* 1952: 30:Suppl. 2.
  17. LOEB, L.: *Proc. Soc. Exper. Biol. & Med.* 1932:29:1128.
  18. LÖFGREN, L.: (to be published).
  19. MONEY, W. L., KIRSCHNER, L., KRAINSTZ, MERRILL, P., and RAWSON R. W.: *J. Clin. Endocrinol.* 1950:10:1282.
  20. PASCHKIS, K. E., CANTAROW, A., EBERHARD, T., and BOYLE, D.: *Proc. Soc. Exper. Biol. & Med.* 1950:73:116.
  21. PERLA, D., and ROSEN, S. H.: *Arch. Path.* 1935:20:222.
  22. SCOTT, W. J. M.: *J. Exper. Med.* 1928:47:185.
  23. SELYE, H.: *The Physiology and Pathology of Exposure to Stress.* Acta Inc. Medical Publishers, Montreal 1950.
  24. SELYE, H.: *Annual Report on Stress.* Acta Inc. Medical Publishers, Montreal 1951.
  25. SPINELLI, A.: *Bull. Soc. ital. bioch. sper.* 1929:4:937.
  26. TALA, P.: *Acad. Diss., Acta Endocrinol.* 1952:10:Suppl. 9.
  27. UOTILA, U.: *Acta Soc. Med. Fenn. Duodecim* 1940:23A:1.
  28. UOTILA, U., and FRIEDGOOD, H. B.: *Acta Soc. Med. Fenn. Duodecim* 1940:23A:1.
  29. UOTILA, U., and FRIEDGOOD, H. B.: *Ztschr. f. d. ges. exper. Med.* 1943:112:579.
  30. UOTILA, U., and KANNAS, O.: *Acta Endocrinol.* 1952:11:49.
  31. UOTILA, U., and PEKKARINEN, A.: *Acta Endocrinol.* 1951:6:23.
  32. WALPOLE, S. H., VARCO, P. L., CODE, CH. F., and WANGENSTEEN, O. H.: *Proc. Soc. Exper. Biol. & Med.* 1940:44:619.
  33. VOLTERRANI, O., and MARCHIS, E.: *Folia Endocrinol.* 1952:5:189.
  34. WYRNAN, L. C.: *Am. J. Physiol.* 1929:89:356.
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## HEMAGGLUTINATION TITRE OF MUMPS VIRUS AT DIFFERENT TEMPERATURES IN THE PRESENCE OF NORMAL ALLANTOIC FLUID

by

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It is a known fact that hemagglutination with the viruses of the influenza -NDV- mumps group usually occurs at room temperature, and in many cases more readily at refrigerator temperature (2). But it is less well known that the agglutination in some special cases seems to emerge only at temperatures above room temperature. This was ascertained in investigations effected following the fact that in some consecutive experiments to study the effect of the allantoic fluid inhibitor on the mumps virus the results varied for no clearly apparent reason. The titrations were made according to the pattern technique of Salk, with plastic plates on a black-surfaced table; an electric lamp was lighted on the table at varying distances, and from time to time solar radiation was introduced. Recordings showed that the temperature on the surface of the table was raised even as much as 10 degrees centigrade by the artificial or natural radiation. These temperature changes accounted for the variation in the consecutive experiments. The present paper describes more closely the effect of temperature on results of hemagglutination tests with mumps virus.

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## TECHNIQUE

The allantoic fluids containing virus were prepared as earlier described (3). As a rule and unless otherwise mentioned plastic plates have replaced tubes in the titrations (4). The titres have been computed from a twofold dilution series made in a volume of 0.25 cc with phosphatebuffered saline solution as the diluting fluid. The cells employed consisted of 0.5% of fresh chicken cells, 0.25 cc added to each dilution. Readings were taken after 1 hour.

## RESULTS

Table 1 shows the change in the titration results of the normal allantoic fluid and the mumps virus allantoic fluid mixture resulting from raising the temperature from  $+17^{\circ}\text{C}$  to  $+37^{\circ}\text{C}$ . Before titration each mixture was kept at  $+4^{\circ}\text{C}$  for a minimum of half an hour, except otherwise stated. The experiments, apart from that at  $+17^{\circ}\text{C}$ , were carried out in tubes in a water bath the temperature of which did not vary more than  $0.2^{\circ}\text{C}$  in the course of the test. The pipetting, however, was effected at room temperature, upon which the tubes were immediately brought to the temperature in question.

TABLE 1

TITRES AT DIFFERENT TEMPERATURES OF A MIXTURE (9+1) OF NORMAL ALLANTOIC FLUID (OF 11-DAY OLD EGGS) AND OF ALLANTOIC FLUID CONTAINING MUMPS VIRUS (ENDERS STRAIN). THE TITRE OF MUMPS VIRUS ALLANTOIC FLUID USED IN THE EXPERIMENT WAS 1/640. THE TITRES IN THE TABLE ARE INDICATED IN RECIPROCAL OF THE DILUTION AND COMPUTED FOR THE ORIGINAL ALLANTOIC FLUID CONTAINING MUMPS VIRUS.

	Titre					
	40	80	160	320	640	1280
Temperature $+17^{\circ}\text{C}$	—	—	—	—	—	—
$+23.5$	—	—	—	—	—	—
$+28$	+?	+?	+?	+?	+?	—
$+32.5$	+	+	+	+	+	—
$+37$	+	+	+	+	+	—

+? = clear though no complete agglutination

+ = complete agglutination

Table 1 shows that at room temperature the normal allantoic fluid inhibits the agglutination induced by the mumps virus, as shown before by others (1). However, above room temperatures the negative agglutination result of the mixture of allantoic fluid

and mumps virus becomes positive, the range of the positive result in its entirety gradually shifting as the temperature rises from completely negative to completely positive.

From the results it is therefore possible that relatively small temperature changes may have a considerable influence on the agglutination induced by mumps virus in the presence of allantoic fluid inhibitor. An experiment similar to that reported in Table I was carried out, with concurring results, with the so-called Habel strain of mumps virus. It has to be noted, however, that cells from different chickens produce variation in the results. Mixtures of normal allantoic fluid and mumps virus (9+1) may with some cells show a positive agglutination result already at +4°C. On the other hand, hemagglutination can first be seen at 37.0°C with some chicken cells. The borderline temperature between the positive and negative agglutination result seems to vary when cells of different chickens are used. When five chickens and one rooster (all white Leghorns) were investigated, the cells of two chickens and the rooster gave agglutination results similar to Table I. In one case the cells were weakly agglutinated without virus and were discarded. The cells of one chicken gave positive results from +4°C and the cells of the other chicken first showed a positive agglutination series at +37°C under the conditions presented in Table 1.

In the experiments described above the hemagglutination tests

TABLE 2

THE EFFECT OF TEMPERATURE ON HEMAGGLUTINATION TESTS WITH MUMPS VIRUS ALLANTOIC FLUID (M.ALL.FL.) IN THE PRESENCE OF VARYING AMOUNTS OF NORMAL ALLANTOIC FLUID (N.ALL.FL.). THE TITRES AND DILUTIONS IN THE TABLE ARE INDICATED IN RECIPROCAL OF THE DILUTION AND COMPUTED FOR THE ORIGINAL ALLANTOIC FLUID CONTAINING MUMPS VIRUS.

Dilution of m.all.fl. in n.all.fl.	Hemaggl. Titre	
	+4°C Refrigerator	+36°C Incubator <sup>1</sup>
undiluted	1280	1280
1.25	1280	1280
2.5	640	640
5.0	320?	640
10.0	<40	640

? = all positive dilutions show a weak agglutination.

<sup>1</sup> The temperature of fluids on plastic plates in +36°C incubator was according to our measurements about +32°C.

were carried out in the presence of added normal allantoic fluid. Table 2 summarises the results of hemagglutination tests at  $+4^{\circ}\text{C}$  refrigerator and  $+36^{\circ}\text{C}$  incubator when different dilutions of mumps virus allantoic fluid in normal allantoic fluid have been tested. The cells of the rooster mentioned above were used.

Table 2 shows that the effect of the increased temperature on the detecting of hemagglutinins is dependent on the quantitative relationships between infected allantoic fluid and normal allantoic fluid.

When investigating whether the agglutination result can be changed at will from positive to negative and vice versa by moving the agglutination plate from the incubator into the icebox and vice versa, it was observed that the negative result in the icebox will change to positive in the incubator, but the positive series of the incubator will still remain positive when moved into an icebox. (In this type of experiment the settled virus-inhibitor-cell mixtures have first been properly mixed then moved into new temperature and after 15 minutes again mixed. The result was read after one hour as usual.)

The explanation of the variation in the agglutination results at different temperatures possibly is that active mumps virus destroys the allantoic fluid inhibitor during the hemagglutination test at elevated temperature: When normal allantoic fluid and mumps virus allantoic fluid mixture (9+1) was incubated at  $+37^{\circ}\text{C}$ , before the agglutination test at  $+37^{\circ}\text{C}$ , after half an hour no differences in agglutination tests at different temperatures were obtained.

#### DISCUSSION

The results presented show the lability of hemagglutination tests with mumps virus if normal allantoic fluid is present. The experiments have been mainly carried out by titrating the mumps virus preparations directly after icebox storage. This may represent a common technique. Then the agglutination results, however, are dependent on variations in temperature. The different destruction rate of allantoic fluid inhibitor is considered to be the cause of variations. With cells of some chickens the temperature which affects strongly the results might be the temperature of the laboratory.

In the above experiments the inhibitor was added to the allantoic fluid containing mumps virus. This is the kind of situation arrived at when, e.g., in plotting the so-called growth curve, the allantoic fluids are pooled. The pooled fluids may include allantoic fluids containing little or no mumps virus. Practically all virus preparations made of allantoic fluid always include also the component inhibiting hemagglutination; hence the possibility that agglutination will only be obtained above room temperature exists in investigations of both mumps virus and possibly of other similar viruses.

It is to be expected that the agglutination results in different laboratories, when titrating active mumps virus, are easily divergent, if the part played by allantoic fluid inhibitor and cells from different chickens is not taken into consideration.

#### SUMMARY

The effect of increased temperature on agglutination tests with mumps virus is described. In the presence of normal allantoic fluid hemagglutinins, not possible to detect at normal temperatures, can be shown. The mechanism and practical consequences of the effect of increased temperature are discussed. The differences in agglutination tests between the cells of different chickens are emphasised.

#### REFERENCES

1. FLORMAN, A. L.: *Proc. Soc. Exp. Biol. & Med.* 1950:91:403.
  2. OVERMAN, J. R., and FRIEDEWALD, W. F.: *J. Immunology* 1949:62:415.
  3. PENTTINEN, K.: *Ann. med. exper. et biol. Fenniae* 1951:29:344.
  4. SALK, J. E.: *Science* 1948:108:749.
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FROM THE STATE SERUM INSTITUTE AND FROM THE VIRUS LABORATORY,  
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## THE EFFECT OF SONIC TREATMENT ON HEMAGGLUTINATING VIRUSES

### II

THE EFFECT OF SONIC WAVES ON THE HEMAGGLUTINATING PROPERTY  
OF MUMPS VIRUS

by

KARI PENTTINEN<sup>1</sup>

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According to earlier investigations sonic treatment raises to a varying degree the hemagglutinin titre of viruscontaining allantoic fluids (3, 5). Sonic treatment is particularly effective on allantoic fluid containing mumps virus (5). At least one contributory reason that has been demonstrated for the increase in the hemagglutinin titre is the destruction of hemagglutination inhibitors by sonic treatment (5).

In the present paper certain points emerging in connection with the sonic treatment of mumps virus are reported on: a possible fall in the titre during the storage of vaccines made of mumps virus can with the aid of the increasing effect of sonic treatment on the hemagglutination titre be shown to be misleading. The dissimilarity of the growth curves of mumps virus based on hemagglutination titres is described when the values obtained by means of sonic treatment are compared with those obtained by other techniques.

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## EFFECT OF SONIC WAVE TREATMENT ON STORED MUMPS VIRUS VACCINES OF WHICH THE HEMAGGLUTINATION TITRE HAS DECREASED DURING STORAGE.

*Technique.* — The batches of mumps virus vaccine were made as follows: 0.2 ml of  $10^{-3}$  dilution of Enders strain of mumps virus were injected into the allantoic sac of eggs incubated for 7—8 days at  $+38^{\circ}\text{C}$ . After 5—6 days of incubation at  $+36.0^{\circ}\text{C}$  the eggs were cooled and the allantoic fluids collected. The fluid was centrifuged for 10 min. at 1,500 r.p.m. and 0.3% formalin added to the supernatant fluid. After storage for 48 hours at  $+4^{\circ}\text{C}$  the fluid was centrifuged at 15,000 r.p.m. Subsequently the precipitate was suspended in saline solution buffered by phosphate. The treatment of the batches of vaccine in a Raytheon 9 KC sonic oscillator machine was effected as reported earlier (5). The hemagglutination titrations were effected according to the pattern technique of Salk with plastic plates (7). 0.5% chicken cells were used and the readings were taken after one hour. In growth curve experiments the titration at  $36^{\circ}\text{C}$ , in addition to usual room temperature method, was used. The titration in elevated temperature has been shown to detect hemagglutinins, which can not be found in titrations at room temperature (6).

For certain experiments an attempt was made to bring the amount of virus in the vaccine used to hemagglutination titre 1/256 by diluting the concentrated vaccine. The titrations were effected in the way described before at room temperature before sonic treatment. As a preservative the vaccines contained 1/10,000 of merthiolate and 1/1,000 of phenol. The vaccines had to be stored at  $+4^{\circ}\text{C}$  for up to six months before use. The hemagglutinating titres of the vaccines, however, were found to have dropped considerably. A similar observation of the fall in titres of phenolised mumps virus vaccines during storage has been made by Beweridge and Lind (1), and of another type of vaccine by Henle *et al.* (2). With titration after sonic treatment, on the other hand, most of the batches of vaccine revealed titres higher than the original. Table 2 shows the hemagglutination titres of the various batches on titration before and after sonic treatment.

Unfortunately the sonic titres of the vaccine batches were not

TABLE 1

INCREASING EFFECT OF SONIC TREATMENT ON THE HEMAGGLUTINATION TITRE OF STORED MUMPS VACCINES. ORIGINAL TITRE OF VACCINES WITHOUT SONIC TREATMENT 1/256. PERIOD OF STORAGE AT  $+4^{\circ}\text{C}$  1—6 MONTHS. TITRES INDICATED IN RECIPROCAL OF DILUTION.

Hemaggl. Titre	No. of Vaccine Batch								
	2	3	4	5	6	7	8	9	10
Before sonic treatment ..	32	64	64	16	28	32	16	32	32
After sonic treatment ..	1024	1024	512	128	128	512	128	512	1024

determined immediately on preparation; hence the changes are not known. It may be mentioned in this connection that in the preparation of the vaccines sonic treatment seems to be particularly effective in dispersing centrifuging precipitates difficult to make into suspensions.

On the basis of earlier studies the effectiveness of sonic treatment may lead to the consideration that the reduction in the titre of the vaccines during storage, partially at least, is due to the inhibitor of hemagglutination which accompanies the virus during differential centrifuging and is destroyed or deliberated from the virus by sonic treatment.

#### EVALUATION OF THE GROWTH CURVE OF THE MUMPS VIRUS BY MEANS OF DIFFERENT HEMAGGLUTINATION TECHNIQUES

*Technique.* — 0.2 ml of undiluted fresh allantoic fluid containing mumps virus was injected into the allantoic sac of eggs incubated for 9—10 days at  $37.5^{\circ}\text{C}$ . At fixed intervals the allantoic fluids of a number of eggs were collected after refrigeration and combined. After centrifuging (10 min. at 1,500 r.p.m.) hemagglutination titrations were made of each allantoic fluid pool by the techniques reported above.

Fig. 2, like Fig. 1, shows that sonic treatment can demonstrate hemagglutinins where the ordinary technique reveals none. Titrations in an incubator also show hemagglutinins when titrations at room temperature show none. On the other hand, temperature changes in titrations after sonic treatment do not affect the results to any considerable degree.

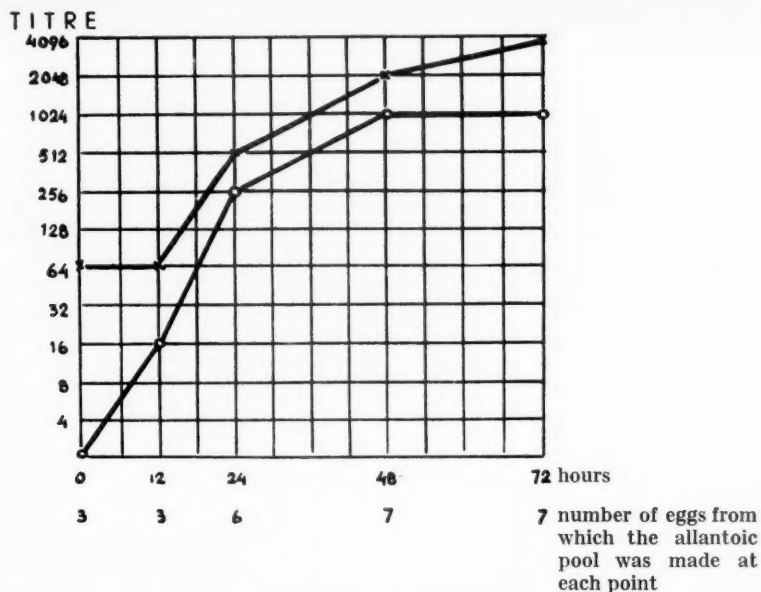


Fig. 1. — Growth curve of mumps virus evaluated by means of hemagglutination. 0-0 titrations at room temperature in the usual way. x-x titrations after sonic treatment of allantoic fluid. The 0 hour indicates that the eggs were placed in the refrigerator immediately after infection. The titres are indicated in reciprocals of dilutions.

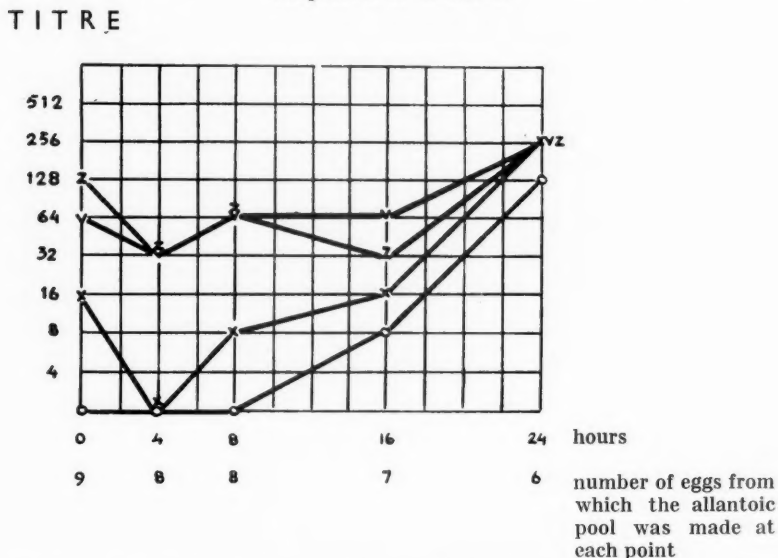


Fig. 2. — Growth curve of mumps virus evaluated by means of hemagglutination. o-o titration at room temperature in the usual way. x-x titrations in an incubator in the usual way. v-v titrations at room temperature after sonic treatment. z-z titrations in an incubator after sonic treatment. The titres are indicated in reciprocals of dilutions. The 0 hour indicates that the eggs were placed in the refrigerator immediately after infection.

## DISCUSSION

It has been shown that the presence of inhibitors causes errors in evaluating the growth curve by hemagglutination technique when influenza virus is in question (4). From the above curves, in which the effect of the inhibitor has been reduced by sonic treatment or by titration at 37°C, this seems also to be the case when mumps virus is in question.

A comparison between the results with sonic treatment and titration effected at a higher temperature seems to indicate that the former is more effective and that after sonic treatment the temperature change does not alter much the results. This in fact is to be expected as sonic treatment has been shown to destroy hemagglutination inhibitors without the presence of virus (5). In agglutination tests with mumps virus from allantoic fluids one has to consider that some variations in technique may alter profoundly the results (6). After sonic treatment of the allantoic fluids, however, the results are more stable.

## SUMMARY

The article reports on changes in the hemagglutination titre of mumps virus preparations as a result of the sonic treatment. It shows that the fall in the titre of mumps virus vaccines during storage may be largely misleading. The original titre of most batches of vaccines has been restored by means of sonic waves. The article also gives the growth curves of mumps virus evaluated by means of different hemagglutination techniques such as titration of allantoic fluid at room temperature, at +36°C and titration after sonic treatment. By means of sonic treatment and in lesser degree by titration at +36°C it has been possible to show hemagglutinins in fluids where ordinary technique shows none. The differences in the effect of increased temperature and of sonic waves are discussed.

## REFERENCES

1. BEWERIDGE, W. J. B., and LIND, P. E.: *Austr. J. Exp. Biol. & Med. Sci* 1947:25:337.
2. HENLE, G., BASHE, W. J. JR, BURGOON, J. S., BURGOON, C. F., HUNT, G. R. JR., and HENLE, W.: *J. Immunology* 1951:66:561.

3. LAZLO, B., and MOLNAR, E.: Kiserletes Orvostudomány 1950:2:357  
Cited after Chem. Abstr.
  4. LIU, O. C., and HENLE, W.: J. Exp. Med. 1951:94:269.
  5. PENTTINEN, K.: Ann. med. exper. et biol. Fenniae 1951:29:344.
  6. PENTTINEN, K.: In press.
  7. SALK, J. E.: Science 1948:108:749.
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## EXPERIMENTAL INDUCTION OF CHRONIC ATROPHIC GASTRITIS WITH VARIOUS TYPES OF METAPLASIA

by

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The literature contains reports of several attempts to produce chronic gastritis experimentally (*e.g.*, 1—3, 13—16, 25). The present paper records the results of some orientating experiments in which the writers tried to induce gastritic alterations with different methods. This paper forms a part of our investigation program in experimental gastric carcinogenesis (4—9, 18, 20—24).

### MATERIAL AND METHODS

The results to be presented now come from a series of three Rhesus monkeys<sup>4</sup>, 39 domestic rabbits and six guinea pigs. (The animals subjected to the same treatment but not included in this report were allowed to live as long as possible.) The monkeys (Nos. 7, 8 and 15) weighed between 2100 and 4800 g. The rabbits weighed between 1400 and 2400 g and were of both sexes. The weight of guinea pigs was about 400 g. All the animals were fed on usual appropriate diet and were given water *ad libitum*.

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<sup>4</sup> In addition, gastrobiopsies were from time to time taken from 20 others control monkeys in order to follow physiologic alterations in the gastric mucosa (together with P. Ermala, M.D., and M. Siurala, M.D.).

One of the monkeys (No. 7) was operated upon in the following manner to induce uncontrolled reflux of the intestinal content: laparotomy under ether anesthesia. The small bowel was sectioned approximately 25 cm below the pylorus, and the oral part was anastomosed with the fundal part of the stomach, while the distal part was connected with the antrum close to the pylorus.

In order to induce, in particular, connective tissue changes the compound used in some of our series for «sclerosing» was a mixture employed in this country for the treatment of varicose veins (Varietol, Medica). One cc contained 0.1 g diethylamine oleinate. The compound was injected at laparotomy intramurally into the gastric wall with the animal under ether anesthesia. The dose varied from 3.0 to 6.0 cc. Sometimes the injection was repeated two to three times. An attempt was made to do the injections so that the compound should, if possible, remain local and not be diffusely spread. The animals, in general, tolerated the injection well, at least the first of them.

One monkey (No. 15) and 19 rabbits (No. 5, 7, 9, 12—15, 17, 20, 22, 27, 30—33, 38, 39, 41 and 51) were fed six days a week by means of a stomach-tube 30 and 10 cc, respectively, a day of «artificial intestinal content» (A.I.C.). This contained bile constituents, neutral fats and lipase, in appropriate proportions. To this was added 0.5:1000 of 9,10-dimethyl-1,2-benzanthracene (Light & Co., London), as a known carcinogen, and per 100 cc of this mixture one teaspoonful of heated lard and the same amount of heated cholesterol, both heated in an open iron pot at 320° C for 2½ hours, as possible carcinogens (cf. 2, 17). The mixture was stirred for 12 to 24 hours at 37° C in a water-bath. [The hydrocarbon appears in the various components of A.I.C. partly dissolved in fats and partly as fine emulsions. However, it is important that a considerable amount of the hydrocarbon is *solubilized* by the micelles of the association colloids (cf. our earlier papers, especially 4—7).] The surface-tension of this mixture was about 30 dynes/cm at room-temperature. Carcinogenic hydrocarbons solubilized by these lipophilic-hydrophilic compounds are easily penetrated into the unchanged gastric glandular mucosa (4, 9, 18, 22, and unpublished data<sup>5</sup>). A full account of the chemical and physico-chemical data will be given elsewhere.

<sup>5</sup> Together with P. Ermala, M.D., and P. Ekwall, Ph. D.

Changes in the gastric mucosa in monkeys were followed by means of gastrobiopsies (24).

The animals were killed with ether or chloroform when their condition grew poor. The conclusions drawn from the histologic findings have been based only on the cases where autopsy was performed immediately after death. The stomach was opened with scissors along the greater curvature, washed in aqueous formaldehyde solution, pinned on stiff paper and fixed in the same solution. The slides were stained routinely with van Gieson, and hematoxylin-eosin. If required, the following techniques were employed: Bauer-Feulgen, Gomori, Foot's stain for reticulin. In the description and comparison of the pathologic findings, Schindler's (19) classification and nomenclature were followed.

## RESULTS

### EXPERIMENTS WITH RHESUS MONKEYS

*Control Animals.* — For this purpose 20 adult healthy Rhesus monkeys were used. Gastrobiopsies were taken from time to time during one year. No noteworthy alterations were seen in the gastric mucosa of the untreated animals.

*Monkey No. 15.* — Male, weight 3 340 g. — On May 4, 1953, laparotomy was performed and 3.5 cc of the sclerosing compound was injected intramurally into the stomach wall. Biopsy (No. 500) from the middle of the stomach body showed fairly normal gastric mucosa. (Fig. 1.) — On May 22, 1953, gastrobiopsy was carried out. (No. 512). No mentionable alterations were seen. On August 12, 1953, laparotomy was again done and 4 cc of the sclerosing compound injected intramurally and on September 7, 1953, 5 cc of the sclerosing compound was injected in the same way.

From August 19 to Oct. 3, 1953, the animal was fed by a stomach tube the A.I.C.-mixture with carcinogens, all in all 33 times. The weight decreased from 3 340 to 2 750 g. The animal was killed on Oct. 5, 1953. At autopsy the gastric mucosa, especially in the body of the stomach, was seen to be polypous or full of small nodules, some of which were stilted, reddish and easily bleeding. In addition, there were three small areas with surrounding wall-like edges (Fig. 2). In the mesentery there was a mass the size of a small apple and microscopically exhibiting signs of chronic non-specific inflammation.

In all regions where macroscopic alterations were noted also microscopically changes of various grades were seen (Figs. 3—4). Changes were found partly in the mucosa partly in the submucosa as well as in the tunica muscularis. In the former the principal gastric glands had nearly completely disappeared giving way to great cells with a cytoplasm staining positively with Bauer-Feulgen's stain. The same type of epithelium covered the

mucosal folds and the surface, as well as the tubules. In some places there were cystic formations which appeared to grow immediately upon the altered muscularis mucosae. Also the epithelium covering the inner surface of these formations was of the above mentioned structure. In a few tubules and cyst-formations covered with mucous epithelium there were scattered areas with altered cells immediately adjacent of the basal membrane. The type of the cells cannot be defined with certainty. The interstitial tissues were in some places edematous, in others infiltrated with round cells. In the lamina propria, muscularis mucosae and tunica muscularis there were great alterations; together these had a thickness of several times the normal, they were fibrotic, and blood vessels were scarce. The macroscopically and microscopically nodular appearance of the mucosal surface depended in part on the fibrous condition of the stomach wall, and partly on hyperplasia of the mucous epithelium.

*Monkey No. 7.* — Male, weight 3 200 g. — On April 15, 1953, laparotomy, gastro-entero-anastomosis and biopsy were performed (No. 600). The mucosa was normal. — On April 24, 1953, gastrobiopsy was done (No. 601). The surface epithelium was normal, the pits were somewhat elongated, the number of chief and parietal cells was reduced; instead there were chiefly mucous cells. — On May 9, 1953, gastrobiopsy was again done (No. 602). The microscopic appearance was practically the same as above. — On May 22, 1953, gastrobiopsy was performed (No. 610). Many alterations were found, there was considerable branching of the tubules, the glands were elongated to tubelike structures. The cell borders were poorly defined, the mucus in places missing. The pits were nearly normal. — On May 27, 1953, laparotomy and intramural injection of 3.4 cc sclerosing compound was performed.

On June 16, 1953, gastrobiopsy was done (No. 611). The mucosa was definitely thin, the pits slightly elongated; only a few chief and parietal cells were left. Considerable amount of inflammatory cells, predominantly round cells were seen. — On July 1, 1953, laparotomy and biopsy were again done (No. 618). Intense proliferation of pits and surface epithelium was noted. Chief and parietal cells were practically completely missing; in their place grew odd cells which did not correspond to any cell-type in the normal mucosa of this same region of the stomach. In the deeper layers were cells which were not mucous. These stained intensely, the borders were poorly defined and the cells reminded to some extent of intestinal ones, but there were no Paneth's cells and no goblet cells. Several cyst-formations were seen. Both the membrana basalis and the lamina propria were collagenous in the areas of the greatest alterations. The surface epithelium resembled that previously described. In the outer layers of the mucosa abundant infiltration with inflammatory cells was noted. The animal's general condition declined steadily. The animal died on August 2, 1953. Macroscopically low noduli were seen in the body of the stomach. The microscopical picture was as above. (Cf. Figs. 5 and 6).

*Monkey No. 8.* — Female, weight 2 540 g. — On April 4, 1953,

laparotomy and intramural injection of 4 cc sclerosing compound was carried out. Biopsy (No. 507) showed a normal mucosa. — On April 22, 1953, gastrobiopsy was again done (No. 510). The mucosa was fairly normal. — On June 26, 1953, laparotomy was again done and 2 cc of the sclerosing compound was injected intramurally and biopsy performed (No. 550). The mucosa was thin, the pits elongated and the surface epithelium of normal appearance. Chief cells were not found with certainty and very few parietal cells were seen, instead there were cells of a «transitional» type which may be of mucous nature. In the outer layers of the mucosa there was abundant round cell infiltration. The connective tissue of the lamina propria stained poorly. The muscularis mucosae was thickened.

As the animal's condition grew worse, it was killed with ether on August 3, 1953. Macroscopically the mucosa was (perhaps) somewhat thicker than usual, but no noduli could be seen. Microscopic examination of the mucosa showed a picture resembling that seen in monkey No. 7. The capillaries were in several places collapsed, the veins of the tunica propria contained only little blood. The connective tissue was clearly increased and somewhat collagenous.

*Comment.* — Three cases of adult Rhesus monkeys are reported in which it has been possible to follow the gradual development of gastritic changes in a previously normal gastric wall. The biopsy specimens were comparable, as they were been taken from corresponding areas of one and the same animal. One can observe the normal mucosa change into gastritic, ending gradually in complete disappearance of the glandular apparatus with consecutive epithelial metaplasia. The microscopic alterations correspond to the findings in the same lesions in man.

In the first case (monkey No. 15), in which sclerosing compound was injected twice and the animal was fed with A.I.C. containing carcinogens, there was a strong proliferation and hyperplasia of the mucous cells, and collagenization of the stomach wall. The terminal condition was an atrophic gastritis inasmuch as the glandular apparatus had disappeared, and a hyperplastic («tumor-simulating») gastritis because the mucous cells proliferated intensely and the collagenized connective tissue stroma produced various nodular appearances.

In the second case (monkey No. 7), in which gastro-entero-anastomosis was made and sclerosing compound was injected once, as well as in the third case (monkey No. 8), in which sclerosing compound was injected twice, there was a change of a normal mucosa in the direction of disappearance of the normal glandular

apparatus and proliferation of atypical cells (called here «transitional» cells). These cells did not resemble any cellular element in the normal gastric mucosa. The type of alteration in question was a beginning atrophic gastritis because the typical cells were decreased in number; on the other hand there were also metaplastic alterations because the new cells were strange to the normal mucosa.

#### EXPERIMENTS WITH RABBITS

Of the 39 rabbits of the series 19 were treated with sclerosing compound and fed with A.I.C. containing carcinogens. The other animals were injected with sclerosino compound a one.

*Rabbit No. 20.* Weight 2 000 g. — On July 30, 1953, laparotomy was carried out. 3 cc of the sclerosing compound were injected intramurally. The animal died on Aug. 8, 1953 (about one week after the operation).

Macroscopically the mucosa was edematous, the folds were flattened. Microscopically the surface epithelium proliferated in several places and there were fairly abundant vacuoles. The pits were somewhat elongated in a corkscrew-like fashion (Fig. 10), the mucus production was increased and some goblet cells were seen. In some parts the edema extended through all layers of the mucosa. As a result of this the gastric glands were pushed far apart from each other and one got a clear view of leaf-vein-like arterioles which radiated from the elements in the muscularis mucosae up to the surface. Some of the arterioles contained blood, most of them being collapsed either only in some zones or over their whole course (Fig. 11). The collapsed arterioles being empty resembled muscle fibers. Slight changes could also be seen in the muscularis mucosae.

*Rabbit No. 1.* — Weight about 2 400 g. — On April 4, 1953, laparotomy and intramural injection of 6 cc sclerosing compound were performed. The animal died on May 12, 1953 (5—6 weeks after the operation). Macroscopically the wall of the stomach was thick and firm. On its mucosal surface was reddish, flat or stilated noduli of varying size and placed close to each other (Fig. 7). They did not bleed easily. These changes were found only in the regions where the injection was made.

The microscopic appearance varied in different areas consisting partly of «pseudopolypous» alterations. (Fig. 8). There was scarcely proliferation of the surface epithelium, vacuoles in several cells and leucocyte diapedesis. In several of the polypes the glandular apparatus was considerably reduced or missing. The interstitial tissue was in some places very edematous and strongly infiltrated with plasma and round cells. Where the actual glandular epithelium was missing mucous cells grew. In some parts were seen cyst-formations of different size and shape covered with cells staining positively with Bauer-Feulgen's stain. This could be seen especially in the outer layers of the mucosa. In several parts of the lamina propria there was a

tremendous collagenosis extending in the form of broad bands up to the surface (Fig. 9). The pits were elongated and partly irregularly formed. In some parts the glands looked normal, in others they had disappeared. Also in these areas there were cyst-formations. In some places the glands seemed to penetrate the muscularis mucosae. However, serial sections showed that the picture arose from enclosure of glands in a net of collagenous connective tissue and not from an active growth of the epithelial elements. The lamina propria, muscularis mucosae and tunica muscularis of the altered areas exhibited intense fibrosis.

*Rabbit No. 11.* — Weight 1950 g. — On June 6, 1953, laparotomy and intramural injection of 3.5 cc sclerosing compound was carried out. The animal died on July 22, 1953 (about 7 weeks after the operation).

Macroscopically, corresponding to the treated zone, the mucosa was covered with fine nodules which were located along the thickened folds. Some of the nodules bled easily, some appeared papillomatous.

The microscopic findings resembled in many areas the changes in the previous cases. But, in addition, the mucosa was greatly thickened in this case owing to the strong glandular hyperplasia. The blood vessels of the mucosa showed the same changes as in the earlier cases.

*Rabbit No. 5.* — Weight 2350 g. — On June 6, 1953, laparotomy and intramural injection of 3 cc sclerosing compound was performed. — On August 28, 1953, laparotomy was again done and 4 cc of the sclerosing compound was injected intramurally. — From August 4 to Oct. 4, 1953, the animal was fed by stomach tube the A.I.C. with carcinogen, all in all 48 times. — The animal died on Oct. 5, 1953 (about 17 weeks after the first operation).

Macroscopically the stomach wall was firm and thickened. The mucous surface appeared normal.

The microscopic examination revealed great alterations both in the surface epithelium, the actual glandular apparatus and connective tissue. The surface epithelium showed considerable proliferation all over, and the cells contained many vacuoles. Mucous cell proliferation was seen chiefly in areas where the corresponding connective tissue was collagenized. In the interstitial tissues, especially in the basal zone of the mucosa, a severe edema was seen pushing the glands upwards and apart from each other. The membrana propria, muscularis mucosae and tunica muscularis showed a distinct change in the direction of fibrosis.

*Other Rabbits* (not recorded above). — Among these great alterations were found in rabbits 2, 4, 6, 7, 13, 14, 15, 30, 48 and 51.

Up to now there has been approximately the same degree of changes found in both the fed and the non fed groups of rabbits.

The changes varied in intensity, appearance and distribution from case to case. In principle, all the above mentioned alterations were seen. In several cases the alterations were apparent only on microscopic examination while the macroscopic appearance was only slightly abnormal. The changes were, in general, greater with increasing dose of the sclerosing compound (doses up to 6 cc), but also smaller doses (about 3 cc) appeared to effective

if the compound remained locally and did not spread. In this connection we want to note the striking resistance of the stomach wall as compared with the skin where injection of 0.5 cc already caused a necrotic area. In many cases adenomatous growth was found also, deep in the submucosa. Serial sections again showed in these cases enclosure of the glandular apparatus in a net of fibrotic connective tissue.

*Comment.* — Some illustrative cases of our series are presented. Microscopically the cases had in common a great increase in and alteration of the connective tissue, often with fore-going or simultaneous circulatory disturbances. It was remarkable that gastritic alterations of different types could be induced in one and the same animal with the same technique. Morphologically these changes correspond to the various gastritic conditions encountered in man.

#### EXPERIMENTS WITH GUINEA PIGS

*Guinea Pig No. 5.* — The weight of the guinea pig was about 400 g. — On April 27, 1953, laparotomy and intramural injection of 3.0 cc sclerosing compound was carried out. The animal died on June 26, 1953.

Macroscopically the stomach wall appears slightly thicker than normal on the site of the injection.

Microscopically the greater part of the mucosa as well as the other parts of the gastric wall are normal, but on the site of injection the lamina propria and muscularis mucosae are damaged. On these parts there are cyst-formations of different size and shape, covered with mucus-secreting cells, and some cells resembling somewhat the goblet cells.

*Comment.* — A typical strictly local alteration of the gastric mucosa induced in a guinea pig is presented. Corresponding changes were found in the other guinea pigs treated with intramural injection of the same compound.

#### DISCUSSION

The present experiment was based on an idea expressed *e.g.*, already by Thiersch about the begin of this century and which also one of us has followed (28, 29); that various changes in the connective tissue — if sufficiently intensive — must in some way, directly or together with some at the same time acting noxious agent(s), influence the corresponding parenchyma.

It appeared that various types of both atrophic and hyperplastic («tumor-forming gastritis»), as well as metaplastic alterations, could be induced in the gastric mucosa in animals hitherto

treated (monkeys, domestic rabbits, guinea pigs). It was also possible to induce a variety of changes in one and the same animal. Further, metaplastic changes in monkeys developed in a mucosa considered normal before the experiment was started. The induced conditions have their morphologic equivalents in man. The resulting gastritic alterations were morphologically similar both in the fed group (19 animals) and in the non-fed group (20 animals) of rabbits. Therefore it seems presumable that the changes were caused by injecting the non-carcinogenic compound intramurally into the gastric wall.

It is not possible from these experiments to say whether the epithelial changes are secondary to the complex lesion of the connective tissue or whether both alterations run parallel: possibly changes in the connective tissue matrix play a much greater role for the behaviour of the mucosal epithelium (especially the mucous one) than hitherto considered, but no conclusions in this respect can yet be drawn.

Some of the alterations induced in the present way, without the use of any previously known carcinogen, also resemble certain stages of development of new growth induced by intramural injection of the carcinogen (10, 12, 26—27), by oral administration of motor lubricating oil (14—15), and are somewhat similar with the alterations seen in autotransplantation of the gastric mucosa into the abdominal wall (11). It seems possible that some of the so-called initial stages in the action of carcinogens perhaps could be of non-specific nature. In the same direction point also our investigations concerning the effect («solvent effect», cf. 21, 23) on the skin of certain lipophilic-hydrophilic vehicles, as well as the anaplasias and metaplasias which we have induced in the colon of rats by the use of the sclerosing compounds (unpublished experiments).

The reported gastritic alterations are readily reproducible. The experiments, which so far have been of orientating nature, are continued with the above mentioned techniques in combination with feeding of various known carcinogens solubilized in stable and homogenous aqueous solutions of certain known lipophilic-hydrophilic compounds.

## SUMMARY

It is possible to induce experimentally various types of gastritic alterations in animals of different species (monkeys, domestic rabbits, guinea pigs) by injecting diethylamine oleinate intramurally into the stomach wall. Gastrobiopsies showed that the gastric mucosa in the monkeys was normal before the experiment was started, and the development of the gastritic lesions could in this way be followed closely.

The results of these investigations together show, in the writers' opinion, that there may exist an intimate relationship between the alterations of the connective tissue elements and especially the mucous-secreting cells of the epithelium.

The investigation continues and most of the animals are allowed to live as long as possible.

## REFERENCES

- 1) BARRETT, M. K.: J. Nat. Cancer Inst. 1946:7:127.
- 2) BECK, S., and PEACOCK, P. R.: Brit. Med. J. 1941:2:81.
- 3) DOUGLAS, D. M., GHENT, W. R., and ROWLANDS, S.: Lancet 1950: 1:1035.
- 4) EKWALL, P., ERMALA, P., SETÄLÄ, K., and SJÖBLOM, L.: Cancer Research 1951:11:758.
- 5) EKWALL, P., and SETÄLÄ, K.: Acta Chem. Scandinav. 1948:2:733.
- 6) EKWALL, P., and SETÄLÄ, K.: Acta Unio Internat. Contre le Cancer 1950:7:120.
- 7) EKWALL, P., SETÄLÄ, K., and SJÖBLOM, L.: Acta Chem. Scandinav. 1951:5:175.
- 8) EKWALL, P., and SETÄLÄ, K.: Acta Path. et Microbiol. Scandinav. 1949:26:795.
- 9) ERMALA, P., SETÄLÄ, K., and EKWALL, P.: Cancer Research 1951: 11:753.
- 10) HARE, W. V., STEWART, H. L., BENNETT, J. G., and LORENZ, E.: J. Nat. Cancer Inst. 1952:12:1019.
- 11) HOWES, E. L.: J. Nat. Cancer Inst. 1949:10:377.
- 12) HOWES, E. L., and OLIVEIRA, J. R. DE: Cancer Research 1948:8:419.
- 13) IVY, A. C.: J. Nat. Cancer Inst. 1945:5:313.
- 14) LUSHBAUGH, C. C.: J. Nat. Cancer Inst. 1947:7:315.
- 15) LUSHBAUGH, C. C.: Cancer Research. 1949:9:385.
- 16) MOORE, G. E., SMITH, G. A., and BRACHNEY, E. L.: J. Nat. Cancer Inst. 1953:13:963.
- 17) PEACOCK, P. R., BECK, S., and CHALMERS, J. G.: J. Nat. Cancer Inst. 1953:13:931.

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- 18) SAXÉN, E., EKWALL, P., and SETÄLÄ, K.: *Acta Unio Internat. Contra Cancrum* 1950:7:156, and 1951:7:423.
  - 19) SCHINDLER, R.: *Gastritis*. W. HEINEMANN LTD, London 1947.
  - 20) SETÄLÄ, K.: *Ann. med. exper. et biol. Fenniae* 1948:26:126.
  - 21) SETÄLÄ, K.: *Acta Path. et Microbiol. Scandinav.* 1949:26:280.
  - 22) SETÄLÄ, K., and EKWALL, P.: *Science* 1950:112:229.
  - 23) SETÄLÄ, K., and EKWALL, P.: *Acta Unio Internat. Contra Cancrum* 1950:7:160.
  - 24) SETÄLÄ, K., ERMALA, P., and SIURALA, M.: *J. Nat. Cancer Inst.* 1953:14:141.
  - 25) SIMON, N.: *Science* 1949:109:563.
  - 26) STEWART, H. L., HARE, W. V., and BENNETT, J. G.: *J. Nat. Cancer Inst.* 1953:13:105.
  - 27) STEWART, H. L., HARE, W. V., LORENZ, E., and BENNETT, J. G.: *J. Nat. Cancer Inst.* 1949:10:359.
  - 28) WANGEL, G.: *Rep. tenth Congress Scandinavian Neurologists* 1948:88.
  - 29) WANGEL, G.: Unpublished data.
-



Fig. 1. — Monkey No. 15. Biopsy (No. 500) before the treatment May 4, 1953. From the middle of the body. Fairly normal gastric mucosa. Hematoxylin and eosin. Medium magnification.

Fig. 2. — Monkey No. 15. Gross specimen obtained at autopsy on Oct. 5, 1953. Chronic atrophic — hyperplastic, nodular gastritis.

Figs. 3 and 4. — Monkey No. 15. Tissue specimens from the stomach seen in Fig. 2. The principal gastric glands are disappeared. The cells are stained positively with the Bauer-Feulgen's technique. Note the tremendous fibrosis or collagenosis and the enormous thickening of the gastric wall. Hematoxylin-van Gieson. Medium magnification.

Fig. 5. — Monkey No. 7. This section was obtained by biopsy (No. 618) July 1, 1953. Note the «corkscrew»-like elongation of the pits. Hematoxylin-van Gieson. Medium magnification.

Fig. 6. — Monkey No. 7. High-power magnification. Partly «transitional»-type of epithelium.

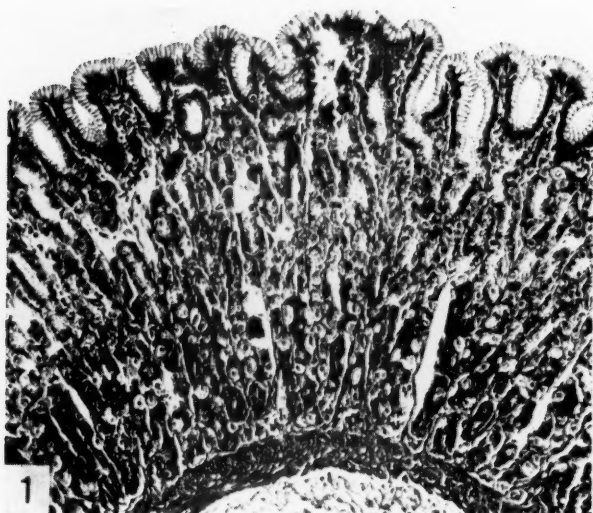
Fig. 7. — Rabbit No. 1. Gross specimen obtained at autopsy on May 12, 1953, *i.e.*, about 5 ½ weeks after the intramural injection of the sclerosing compound. Chronic tumor-forming («polypoid») gastritis. The nodulation is *not* a postmortem formation of areolae gastricae. This animal was not fed with any carcinogen or artificial intestinal content.

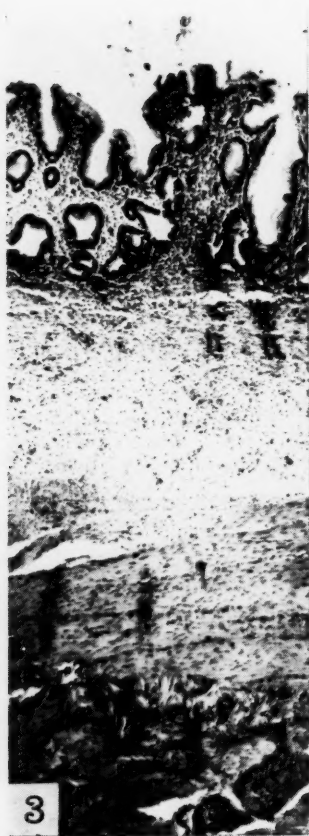
Fig. 8. — Rabbit No. 1. This section was obtained at autopsy (*cf.* Fig. 7). Polyposis of the gastric mucosa. Most of the principal glands have disappeared. Extensive vacuolation in the irregular epithelium. Moderately round cell infiltration. Edema of the mucosa. Hematoxylin-van Gieson. Low-power magnification.

Fig. 9. — Rabbit No. 1. (*cf.* Figs. 7 and 8). Strong interstitial fibrosis. The number of gastric glands is decreased. Hematoxylin-van Gieson. Low-power magnification.

Fig. 10. — Rabbit No. 20. This specimen was obtained at autopsy on Aug. 8, 1953, about one week after treatment with sclerosing compound. Note the «corkscrew»-like elongation of the pits.

Fig. 11. — Rabbit No. 20. High-power magnification. It appears that the structures which in some places perhaps might resemble muscle fibers are arterioles; some of which are collapsed, others containing a few clearly visible erythrocytes. Hematoxylin-van Gieson.



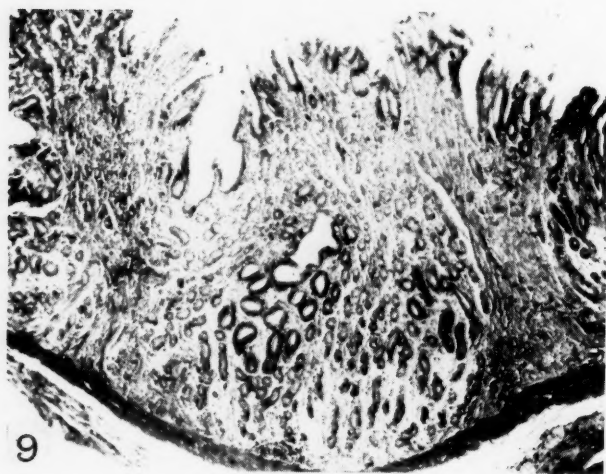
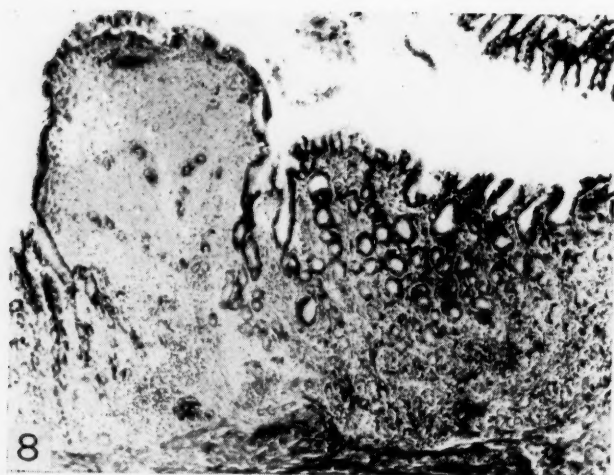


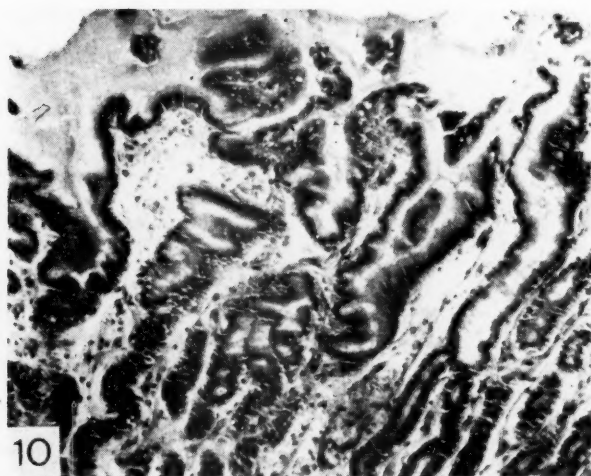


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## EXPERIMENTAL LIVER CIRRHOSIS

INDUCED IN RABBITS FED WITH 9,10-DIMETHYL-1,2-BENZANTHRA-  
CENE, HEATED FATS AND BILE CONSTITUENTS, ALONE AND IN COMBI-  
NATION WITH THE INJECTION OF A SCLEROSING AGENT

by

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WANGEL

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In the course of our present long-term experiments with feeding of 9,10-dimethyl-1,2-benzanthracene, heated fats, lipase and bile constituents solubilized by aqueous solutions (4) (here called artificial intestinal content and carcinogen), we have been astonished by the regular appearance of liver cirrhosis in our rabbits. Besides constituting a serious complication to an experiment like ours the induced cirrhosis was unexpected because of the reported resistance of the liver to feeding with carcinogenic hydrocarbons (1,3) [except for the usually very weak 1,2-benzanthracene, reported to produce hepatomas in rats (7)]. Therefore we feel justified in reporting this finding at this stage although the primary experiment is still incomplete.

### THE PRESENT INVESTIGATION

The experiment was made primarily in order to produce changes in the gastric wall which would form a suitable tissue of origin for the induction of experimental gastric cancer<sup>3</sup>. As we paid insufficient attention to the liver at the commencement of our experiment it is not possible to report on the incidence of liver changes in the

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<sup>3</sup> Unpublished data.

whole of our series. But once we noticed the first case with lesions in the liver we found some degree of liver changes in nearly every animal autopsied. Since the animals are involved in a long-term experiment we do not want to sacrifice them all yet. The series where the liver was studied thus consists to date of 34 domestic rabbits, three Rhesus monkeys and three rats. The animals were fed on an appropriate stock diet and given water *ad libitum*. The monkeys had been kept two years in good health on this diet. In other rabbits given the same diet as the rabbits in the experiment no case of liver cirrhosis was noted.

Sixteen rabbits, one monkey and the three rats (group 1) were, in order to induce alterations in the gastric mucosa, operated upon in the following way: by way of laparotomy a compound «V» commonly used for sclerosing varicose veins was injected into the outer layers of the stomach wall. The compound contains per 1 cc, diethylamineoleinate 0.1, benzylalcohol 0.03 and distilled water ad 1 cc. The dose was 3—4 cc for the rabbits, 4—6 cc for the monkeys and 0.5 cc for the rats. In some of the animals the operation was repeated two to three times with an interval of a month. An endeavour was made to avoid the blood vessels and usually the injection was seen to cause a small subserosal nodule of about 1—2 cm in diameter. Sometimes it was evenly distributed. As the blood vessels form a fairly dense network it was not always possible to avoid damaging a vessel, causing a small hematoma. It might be that some of the material was in this way injected directly into a vein.

These animals were only fed the usual stock diet. Some of the animals died within a week of the operation, usually from hemorrhagic gastritis. Most of the animals stood the operation well and when a few of them later succumbed to respiratory infections various gastric changes and sometimes an ulceration were noted in the stomach (unpublished data).

Eighteen rabbits and two monkeys (group 2) were six days a week fed<sup>4</sup> the above-mentioned mixture of artificial intestinal content containing: bile acids, bile acid salts, fatty acids, lipase, and lard and cholesterin heated to 320° C for 2½ hours.<sup>5</sup> To this was added

<sup>4</sup> By means of stomach tube.

<sup>5</sup> The amount of bile constituents arbitrarily corresponded to the amount in normal intestinal content. One teaspoonful each of lard and cholesterin was added per 100 cc of the mixture.

0.5%<sub>00</sub> 9,10-dimethyl-1,2-benzanthracene. The daily dose was 10 cc for the rabbits and 30 cc for the monkeys. Sixteen of these rabbits and the two monkeys were also injected with the sclerosing compound in the above-mentioned way. Thus there remained two rabbits which were only fed the carcinogen mixture and not operated upon. Because of the small number they are not considered as a separate group. The material obtained at the autopsies was fixed in 10% formalin and stained in the routine way with hematoxylin eosin and van Gieson.

#### RESULTS

*Group 1.* — Cases where only the operation was performed.

This group consists as mentioned of 16 rabbits, one monkey and three rats.

Of these animals five rabbits died within two weeks of the operation, the death resulting from hemorrhagic gastritis and perhaps also the great liver changes. The liver showed necrotic areas of varying sizes and a slight increase in the periportal connective tissue. The liver cells were swollen, the sinusoids often empty. In one case the borders between the liver cells had disappeared in several places so that giant cell-like multinuclear masses formed. They often contained some dark-stained homogeneous material. Some of the liver cells were shrunken and dark. Thrombosed sublobular and central veins were often seen, and sometimes central accumulation of fat.

Four rabbits in this group died about one month after the operation. The cause of death was purulent respiratory infection. In three of those the liver showed small intralobular necrotic areas, slight if any increase in the periportal connective tissue which in a few places was infiltrated with round cells. Some of the liver cells contained granular dark brown pigment. One of those cases exhibited only minor changes.

Two rabbits of group 1 lived six weeks after the operation. Their livers showed moderately severe parenchymal lesion with poor staining of the liver cells and central vacuolisation but only few clearly necrotic areas. No increase in connective tissue was noted. The architecture of the liver lobules was perfectly normal.

Five rabbits of group 1 lived eight to thirteen weeks after the operation. Their livers showed no signs of cirrhosis. No necrotic

areas were seen although some of these rabbits had been operated upon twice (injection with the sclerosing compound).

In two cases the livers of the rats exhibited diffuse vacuolisation, in the third only minor changes.

The only monkey in this group was killed three months after the first injection (and one and a half month after the second one). The liver showed some small necrotic intralobular areas infiltrated with round cells. The architecture of the lobules was normal but the cells were swollen and often vacuolated. No increase in the connective tissue was seen. No animal in group 1 showed any sign of cirrhosis of the liver.

*Group 2.*—Animals fed with artificial intestinal content and carcinogen.

This group includes 18 rabbits and two monkeys. Sixteen of these rabbits and the two monkeys were also injected with the sclerosing compound.

Two of the rabbits died within three weeks after the operation of a purulent respiratory infection. The liver showed small necrotic areas and some vacuolisation of the liver cells. Another rabbit died spontaneously five weeks after the operation, the liver exhibiting abundant small intralobular necroses (Fig. 1) some of which were curiously shaped as a sector of the lobule (Fig. 2).

Eleven rabbits died or were killed after two-three months of feeding with the artificial intestinal content and carcinogen (and 2½—4 months after the operation). Of these rabbits seven had a severe liver cirrhosis with ascites and hydrothorax. Their livers were hard, nodular and yellowish-spotted. Microscopically some of these livers also showed small remnants of necrotic areas, but for the most part they only revealed a picture of severe cirrhosis with broad bands of connective tissue between scattered islands of liver parenchyma not forming regular lobules (Fig. 3). In most of the cases the connective tissue increase was apparently diffuse in the lobule both in the form of capillary fibrosis and of increase in the periportal and pericentral connective tissue. There was a seeming increase in the amount of small bile ducts or formations resembling bile ducts (depending on whether one considers them as being formed from bile ducts or from liver cells). The four other rabbits did not have ascites but showed microscopically definite liver cirrhosis.

The two remaining rabbits which have been fed with the carcinogen mixture for 3—4 months are still alive though losing weight.

The two rabbits treated with artificial intestinal content and carcinogen only both exhibited after two and a half months' feeding a picture of severe cirrhosis with ascites. They were killed and the liver found to be macroscopically typical of liver cirrhosis. Microscopically no necrotic areas were found, only an extensive increase in connective tissue, apparently starting as mentioned above diffusely in the lobule. Collagenisation of the walls of the capillaries was also noted. Only scattered islands of parenchyma mostly without lobular construction, were left. No accumulation of fat was noted (Fig. 4 and 5).

Thus, of 15 rabbits which were fed the artificial intestinal content and carcinogen for two months or more, nine had severe liver cirrhosis and four others showed on microscopical examination extensive cirrhotic lesions of the liver.

Of the two monkeys in this group one died immediately after the second operation, of hemorrhagic gastritis, showing a normal liver. The other monkey was sacrificed for the primary experiment two months after the second operation and after one and a half months of feeding. The liver showed swollen, vacuolated liver cells but no cirrhosis.

In many of the rabbits and in one monkey an extensive dilatation of the bile ducts was noted (or formations reminiscent of bile ducts) with formations of cyst-like structures containing a homogeneous often lamellated material (Fig. 6).

#### DISCUSSION

The sclerosing compound used in the present experiment apparently had both a damaging effect directly on the liver cells and an ability (probably through a cell-damaging property) to induce thrombosis of vessels. The changes this compound apparently caused in the liver were massive or focal necrosis of the parenchyma, vacuolisation of the cells, thrombosed veins and toxic changes in the epithelium of the bile ducts. As mentioned, some of the small intralobular necroses had the shape of a sector of the lobule. This is interesting to note as it has been shown that some sectors of

a lobule can be nutritioned solely by branches of the portal vein. The finding does not anyhow justify any conclusions. The above-mentioned effects appeared in practically all the animals treated with the compound. But though five of the rabbits lived for between eight and thirteen weeks after the operation none showed any clear signs of cirrhosis of the liver. The architectural pattern of the liver lobules was intact,

On the other hand, of the 13 rabbits which in addition to the injection were fed for two months with the artificial intestinal content and carcinogen (9,10-dimethyl-1,2-benzanthracene), seven developed within this time severe cirrhosis of the liver and four showed microscopically definite liver cirrhosis. Two are still alive. The three rabbits which died three to five weeks after the start of the feeding showed no clear signs of cirrhosis, probably due to the short time of the feeding.

In addition, the two rabbits which were not treated at all with the sclerosing compound but only fed with the artificial intestinal content and carcinogen both developed severe liver cirrhosis.

Thus out of 15 rabbits fed with the artificial intestinal content and carcinogen for two months or more 13 developed at least microscopically liver cirrhosis in at least two and a half months.

Since the animals formed part of another experiment not planned to produce liver changes, the series was small. But the total lack of cirrhosis in group 1, only injected with the sclerosing compound, and the high amount of liver cirrhosis in group 2 in combination with the fact that the two rabbits only treated with the artificial intestinal content and carcinogen developed cirrhosis showed in our opinion that the liver cirrhosis was due to the feeding with the above-mentioned mixture. The basic diet seemed to be appropriate because among several other rabbits kept on the same diet no liver cirrhosis was found. The amount of various fats included in the feeding mixture is so small that with an otherwise free diet it should in our opinion not exhibit any «fatty» effect on the liver. In earlier experiments where fatty cirrhosis has been produced in livers of rats by feeding heated lard the amount has been about 35% of the diet and the time required about 7 months (6). In our experiment the amount of heated fats was about 250 mg per rabbit per day, and accumulation of fat in the liver cells was very seldom seen. It remains to be shown in additional experiments

whether 9,10-dimethyl-1,2-benzanthracene solubilized in this way is the main cause of the cirrhosis, or whether the in this way treated fats contain some other compound influencing the liver.

A remarkable feature is the pronounced enlargement of the bile ducts filled with mucus-like, homogenous, lamellated material. This could be the result of perhaps a smaller injury to the bile duct or liver cells (a greater injury leading to necrosis) or of the reaction of these cells to connective tissue injury. Very similar pictures are found in the livers of children dying from fibrocystic disease of the pancreas (mucoviscidosis) (2) and in such cases are thought to depend on the production of an abnormal mucus occluding the bile ducts. Similar changes have also been reported in association with induction of liver tumors (5). In our cases the picture has arisen so rapidly that there should be no occlusion of bile ducts because of fibrosis of the connective tissue. As we have found similar changes in other organs (unpublished data) the relation of these experimentally induced conditions to mucoviscidosis will be discussed in a separate report.

#### SUMMARY

The writers report the regular appearance of liver cirrhosis in rabbits fed for 2—2½ months with 9,10-dimethyl-1,2-benzanthracene, bile constituents, lipase and small amounts of heated fats. The cirrhosis was unexpected because of the reported resistance of the liver to carcinogenic hydrocarbons fed by mouth and because the amount of fat was considered too small to exert any damaging effect on the liver. Besides, no fat accumulation in the liver was noted generally. The experiments are being continued in order to bring additional light to the question of liver cirrhosis in rabbits.

# REFERENCES

- 1) BERMAN, CH.: Primary Carcinoma of the Liver. London, Lewis & Co, 1951.
  - 2) BODIAN, M.: Fibrocystic Disease of the Pancreas. London, Heinemann Medical Books, 1952.
  - 3) CLAUDE, A.: Am. J. Cancer 1937:31:100.
  - 4) EKVALL, P., SETÄLÄ, K., and SJÖBLOM, L.: Acta Chem. Scand. 1951: 5:175.
  - 5) FIRMINER, H. I., and MULAY, A. S.: J. Nat. Cancer Inst. 1952:13: 19.
  - 6) MORRIS, H. P., LARSEN, C. D., and LIPPINCOTT, S. W.: J. Nat. Cancer Inst. 1943:4:285.
  - 7) WHITE, F. R., and ESCHENBRENNER, A. B.: J. Nat. Cancer Inst. 1945: 6:19.
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Fig. 1. — Rabbit, died about one month after the operation. In the liver were seen several necrotic areas of various size. Low-power magnification, hematoxylin and van Gieson.

Fig. 2. — Same rabbit as in Fig. 1. A necrotic area shaped as a sector of the lobule is seen. The central vein is in the middle of the picture. Medium magnification, hematoxylin and van Gieson.

Fig. 3. — Section from the liver of a rabbit which died after about 2 months of feeding with the carcinogen mixture. The picture shows the increased amount of connective tissue surrounding abundant small bile-duct-like formations. Low-power magnification, hematoxylin and van Gieson.

Fig. 4. — Section from the liver of another rabbit which died from liver cirrhosis after 2½ months of feeding with the carcinogen mixture. The increased connective tissue surrounds islands of fairly normal liver cells. Low-power magnification, hematoxylin and van Gieson.

Fig. 5. — Higher magnification of the same liver as in Fig. 4.

Fig. 6. — Enlarged bile duct containing mucus-like material. Medium magnification, hematoxylin and van Gieson.

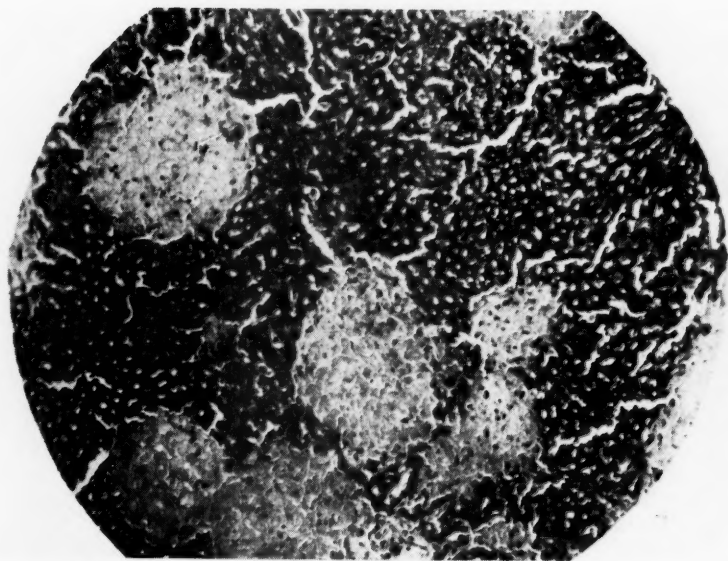


Fig. 1

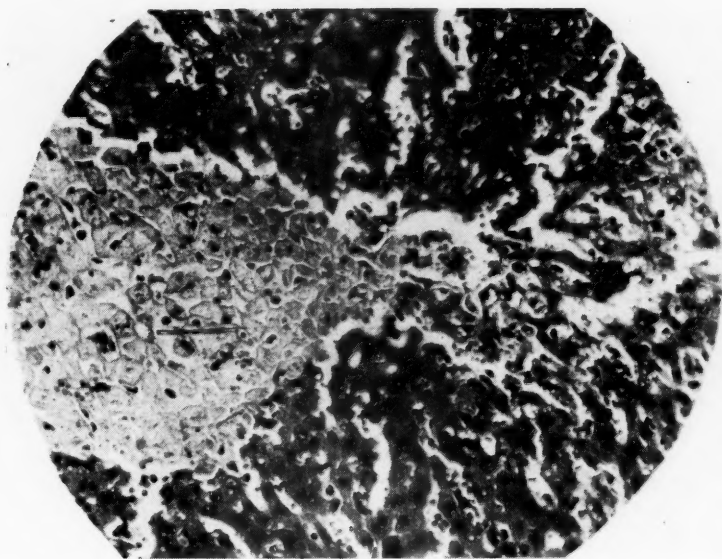


Fig. 2

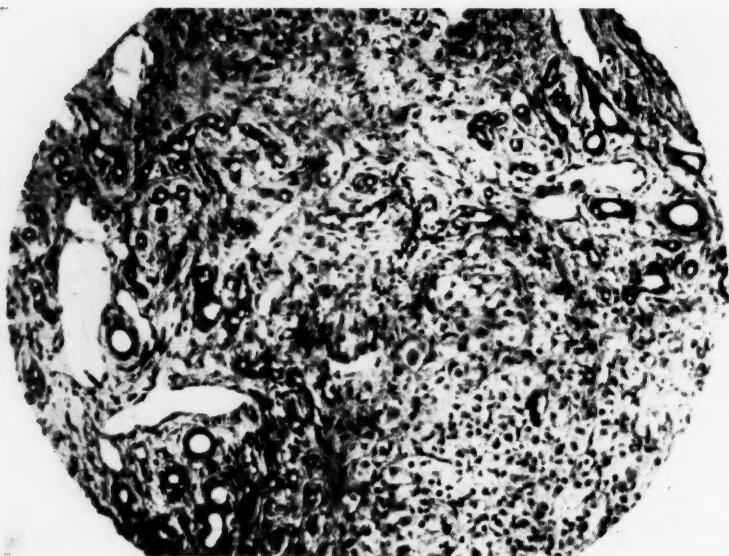


Fig. 3

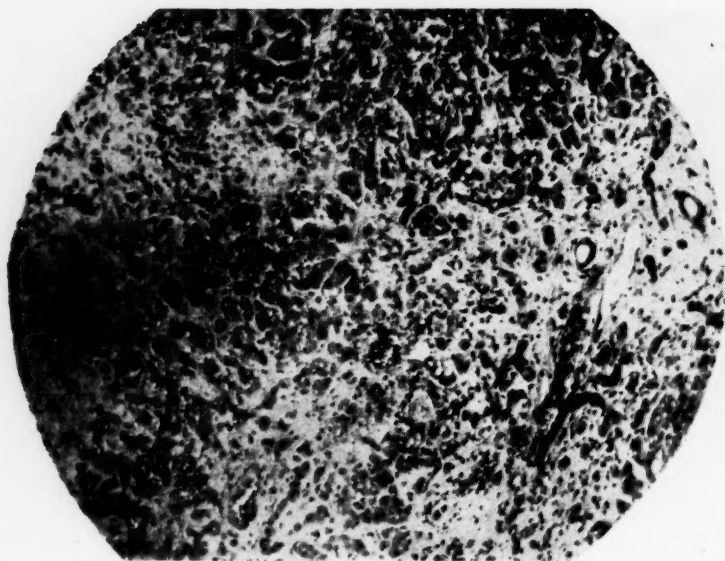


Fig. 4

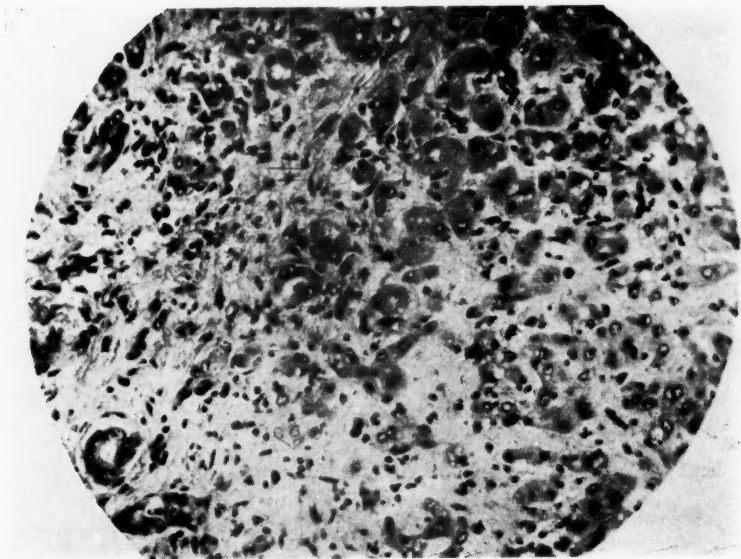


Fig. 5

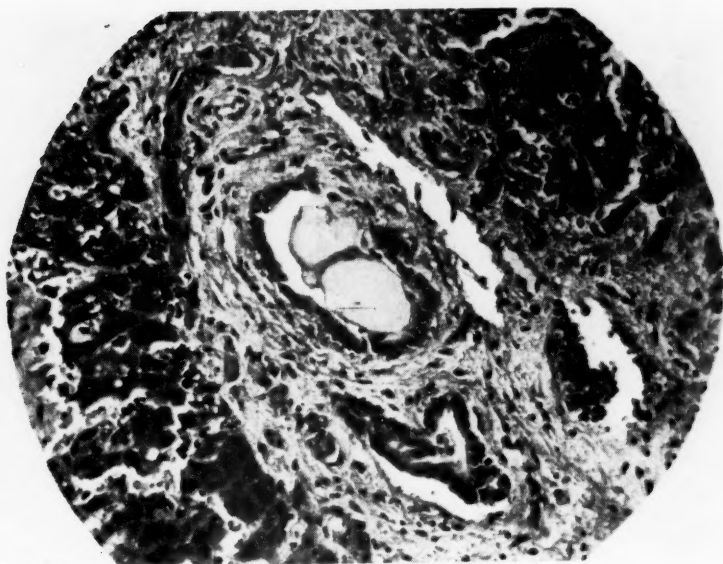
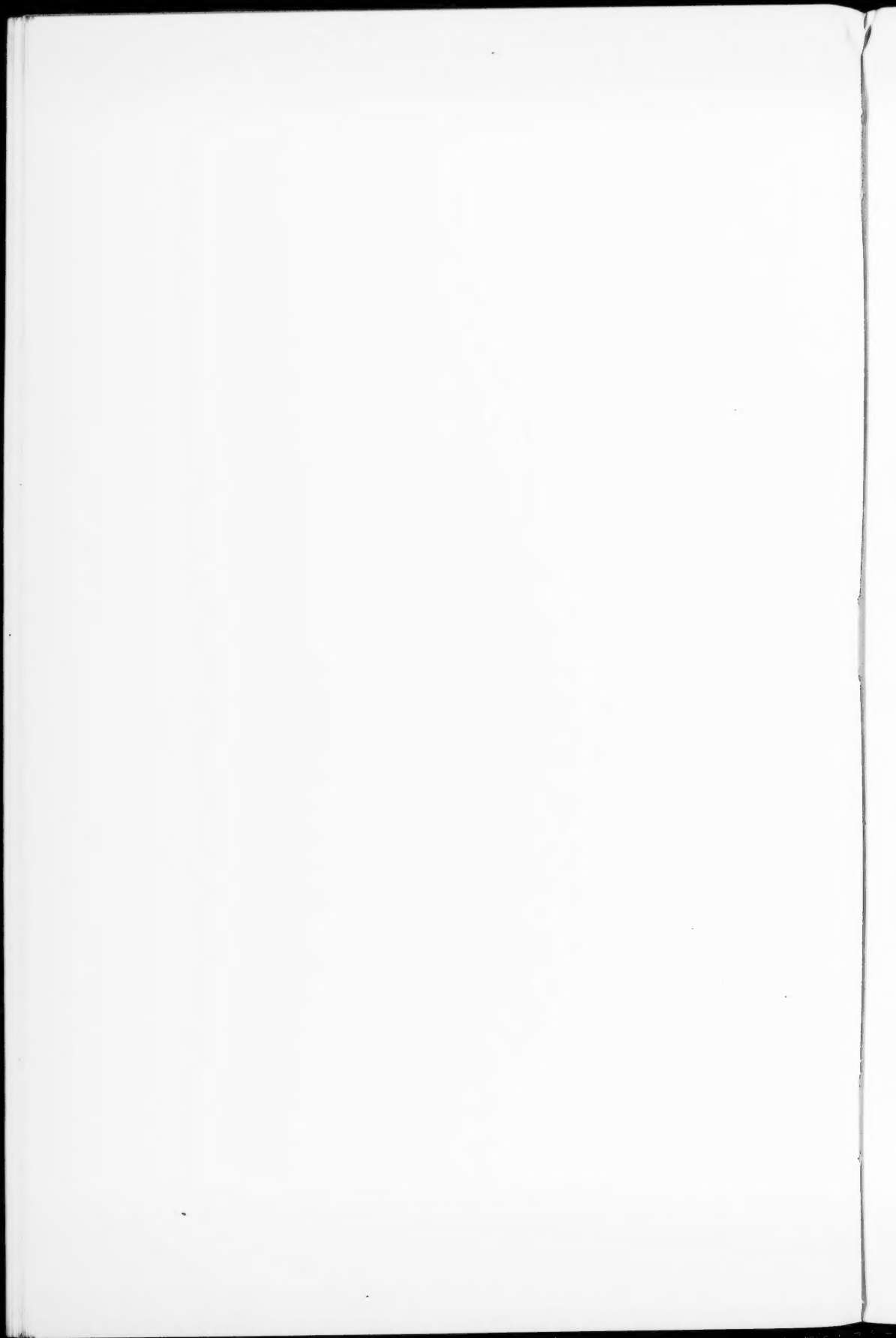


Fig. 6



## EXPERIMENTAL INDUCTION OF CERTAIN METAPLASTIC ALTERATIONS

by

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KAI SETÄLÄ<sup>3</sup>

(Received for publication Jan. 4, 1954)

In a previous study (10), a report of attempts to induce different types of chronic gastritis, we wrote that we have in certain organs of the same animals induced changes morphologically resembling some of the so-called initial stages of cancer development, especially metaplastic alterations.

In the present paper we present those of our observations which seem to us most worth to be noticed. The alterations have been induced in organs or organ groups which embryologically are associated with the mucus-secreting elements, and also in organs in which there is even normally a lively secretion of mucus. These tissues were selected also because the investigation of metaplastic phenomena is easier if the metaplasias induced morphologically clearly differ from the normal tissues of origin.

### MATERIAL AND METHODS

Concerning the general experimental technique of the present investigations, we refer to our previous papers, especially No. 10. The series of the present investigation consisted of four Rhesus monkeys, eight guinea pigs, 32 rabbits and 33 rats.

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<sup>2</sup> Research Assistant in Pathology, aided by a Grant from the Rockefeller Foundation.

<sup>3</sup> Aided by a Grant from the Sigrid Jusélius' Foundation.

## A REVIEW OF THE PRESENT RESULTS

*Changes in the Gastric Mucosa* (Fig. 5). — Diethylamine oleinate was injected in the gastric wall. The essential changes produced in the gastric mucosa have been reported in full details previously (cf. 10).

The series was now greater and showed, in the animals where the condition of the gastric mucosa was followed by repeated gastrobiopsies, a gradual disappearance or atrophy of the principal gastric glands. At the same time — to a increasing degree — only the mucus-secreting elements lived and proliferated in connection with the connective tissue, which was altered in the direction of fibrosis. Further, the alterations induced resembled both the proliferation of the mucus elements and the changes of the connective tissue in cases when carcinogenic hydrocarbons had been injected intramurally into the stomach wall (4, 11, 12). In addition, the results obtained by feeding *e.g.*, of motor lubricating oil (cf. 6—8) reminded morphologically in certain respects of the present results.

Similar alterations were also induced when silk threads were embedded into the gastric wall (unpublished data).

*Changes in the Pancreas* (Figs. 1 and 2). — Since the alterations in the pancreas up to now were most clear and advanced in guinea pig No. 21, this case will be presented in detail.

Guinea pig. No. 21. — Body weight about 400 g. — On April 30, 1953, laparotomy and intramural injection into the ventral and dorsal surfaces of the gastric wall of 4.0 ml of diethylamine oleinate were carried out. — The animal was killed on Aug. 26, 1953, while in good condition. — The pancreas was totally adherent to the dorsal surface of the stomach.

Microscopically advanced alterations were encountered both in the various components of the connective tissue and in the parenchyma, especially in the central parts of the gland. As to the connective tissue, there was, as one extreme, strong proliferation of the fibroblasts with richness of nuclei; as the other extreme there was extensive fibrosis (or collagenosis).

The alterations in the parenchyma were for the greater part degenerative. The excretory components of the pancreas were more severely damaged, whereas the islands of Langerhans were fairly well preserved. In general, the changes were grouped around the damaged

veins as concentric zones with various degrees of alterations; the latter were greatest in the zones nearest to the damaged vessels; and practically taken, all the parenchyma was destroyed here. Only far apart from each other remained some scarce individual atrophic gland cells surrounded by granulation tissue; the zymogen granules were lacking. Outwards from this zone lay the second zone where the destruction of the original elements was less severe. However, all remaining glands were atrophic, and no zymogen granules could be distinguished. The existence of some normally built acini partly surrounded by granulation tissue was characteristic for the third zone; a number of these glands were enlarged. The parenchyma in the fourth zone was, on the whole, of normal architecture.

Most of the acini enlarged in various ways were situated on the border between the third and fourth zones, just along the inner border of the normal parenchyma. In these acini some normal appearing acini containing cells with zymogen granules were seen, and beside these there were lower cells, basophilic and devoid of zymogen granules. On the other hand, in other acini nearly the whole glandular epithelium was basophilic, and only some atrophic remnants of zymogen-containing cells were seen. Most of the cyst-like formations formed in this way were optically empty. In places there appeared — fairly near each other — pancreatic ductuli of various sizes, the lining epithelium of which had partly a striated border; in other places appeared dense accumulation of goblet cells and intensive proliferation of mucus-secreting glands. The latter phenomenon was sometimes so extensive that it dominated the whole microscopic picture (Fig. 2).

*Changes in the Liver* (Figs. 3 and 4). — The changes presented here were induced in rabbits and monkeys by feeding with a mixture of 9,10-dimethyl-1,2-benzanthracene and heated lipids solubilized by aqueous solutions of lipophilic-hydrophilic substances (cf. 10).

At the beginning of the alterations the lining epithelium of the bile ducts was fairly normal; it, however, contained more goblet cells than usual. In addition, the surrounding connective tissue was infiltrated with some inflammatory elements and showed fibroblastic reaction. In the next stage the epithelium was in places desquamated and the ducts enlarged, and there appeared a content which stained eosinophilic with varying intensity. The periductal

connective tissue increased gradually. A little later, the prismatic epithelium of the ducts was completely replaced by atypical elements and the enlarged ducts were filled with lamellated eosinophilic material. At the same time the periductal and interlobular connective tissue increased (cf. 1).

*Changes in the Colon* (Fig. 6). — Anaplastic and metaplastic alterations were produced in several of the treated 33 rats within one to three weeks by injecting diethylamine oleinate intramurally into the wall of the large intestine. — Rat No. 5 is an illustrative case. — On Oct. 5, 1953, 0.5 ml of the compound was injected into the wall of colon about 4 cm aborally from the ileo-coecal valve. — The animal was killed on Oct. 26, 1953. — Microscopically the tunica propria was considerably thickened and the connective tissue was fibrotic, somewhat resembling non-specific granulation tissue. In the area corresponding to the injection site the typical lining goblet cells were completely lacking. Instead there grew basophil, anaplastic cells in one or several layers. These cells were large and intensely staining, their nuclei varied in size and shape and were not always located in the same region of the cell. The borders between the individual cells were not distinct.

#### DISCUSSION

By the local treatment, injection into certain organs of an apparently non-carcinogenic compound, diethylamine oleinate, the writers induced metaplastic alterations especially in the mucous membranes of these organs. Simultaneously, there appeared considerable changes in the corresponding connective tissue stroma.

Some of the changes (especially alterations in the pancreas and liver, and — to some degree — those in the gastric mucosa) resembled changes seen in connection of so-called fibrocystic disease of the pancreas (cf. 2), and sometimes to such an extent that one might speak of a kind of experimental induction of this disease. On the other hand, the various alterations induced by the authors also resembled those obtained by intramural injection of carcinogenic hydrocarbons (*e.g.*, 4, 11, 12), the changes observed in the liver during experimental liver carcinogenesis (*e.g.*, 3, 5, 9), as well as the changes in the stomach caused by feeding motor lubricating oil (6—8). It may be added that it has been possible

to induce certain cutaneous alterations also with some-non-carcinogenic compounds resembling those held specific for the process of cutaneous carcinogenesis (unpublished data; see also our previous papers).

Since the alterations induced by the writers without the use of any known carcinogen (in the stomach, pancreas and large intestine), and, on the other hand, the alterations induced by the use of actual carcinogens had, morphologically, several common features (especially in the initial stages), the question arises which of the changes seen in connection with experimental chemical carcinogenesis are really essential and specific for development of cancer. It is to be remembered that in reality we know very little about the initial stages in the process which leads to malignant transformation of tissues. As a working hypothesis it is, perhaps, possible by the parallel use of the non-carcinogenic and the carcinogenic compounds to split the course of malignant transformation into different components and to some extent throw additional light upon the significance of each of them.

The experiments are to be continued.

#### SUMMARY

The writers produced, in different ways and without the use of any known carcinogen, especially by the intramural injection of an apparently non-carcinogenic compound, metaplastic alterations in the gastric mucosa, pancreas and large intestine. These alterations resembled closely those previously reported as the result of intramural injection of carcinogen in the stomach wall and considered to be specific for carcinogenesis. Thus it might be that the so-called initial stage of carcinogenesis can be split in this way into different components, some of which may be of a non-specific nature.

#### REFERENCES

1. BLUMQUIST, H. E., SETÄLÄ, K. HOLSTI, P., and WANGEL, G.: *Ann. med. exper. et biol. Fenniae* 1954:32:97.
2. BODIAN, M.: *Fibrocystic Disease of the Pancreas: A Congenital Disorder of Mucus Production — Mucosis*. W. Heinemann, London 1952.
3. FIRMINER, H. I., and MULAY, A. S.: *J. Nat. Cancer Inst.* 1952:13:19.

4. HARE, W. V., STEWART, H. L., BENNETT, J. G., and LORENZ, E.: J. Nat. Cancer Inst. 1952:12:1019.
5. LAURENS, J.: and BACON, H.: J. Nat. Cancer Inst. 1952:12:1237.
6. LUSHBAUGH, C. C.: J. Nat. Cancer Inst. 1947:7:313.
7. LUSHBAUGH, C. C.: Cancer Research 1949:9:385.
8. LUSHBAUGH, C. C., and HACKETT, A.: J. Nat. Cancer Inst. 1948:9:159.
9. OPIE, E. L.: J. Exper. Med. 1944:80:231.
10. SETÄLÄ, K., BLOMQUIST, H. E., WANGEL, G., and HOLSTI, P.: Ann. med. exper. et biol. Fenniae 1954:32:78.
11. STEWART, H. L., and LORENZ, E.: J. Nat. Cancer Inst. 1947:7:239.
12. STEWART, H. L., HARE, W. V., and BENNETT, J. G.: J. Nat. Cancer Inst. 1953:13:105.

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Fig. 1. — Guinea pig No. 21. Pancreas. In the upper part of the picture is a completely occluded vein. Most of the parenchyma is destroyed. In the left lower part of the picture are seen some enlarged acini. Hematoxylin — van Gieson. Low-power magnification.

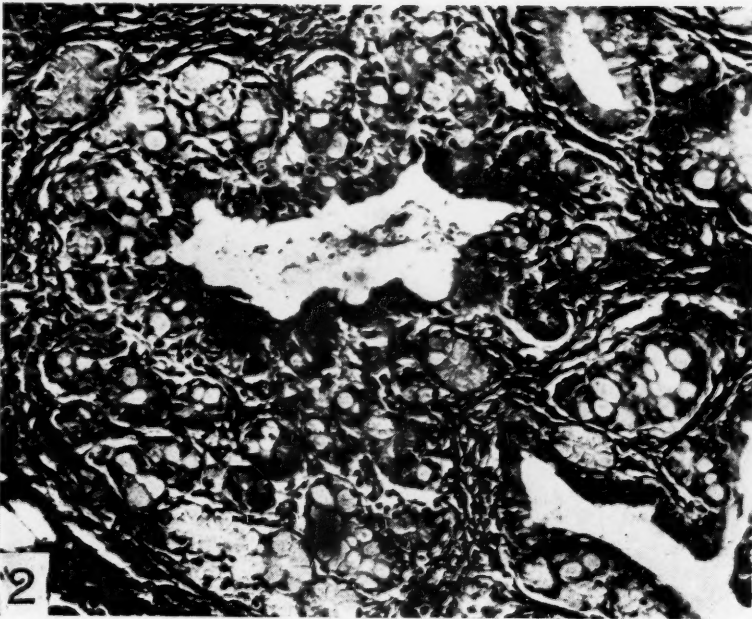
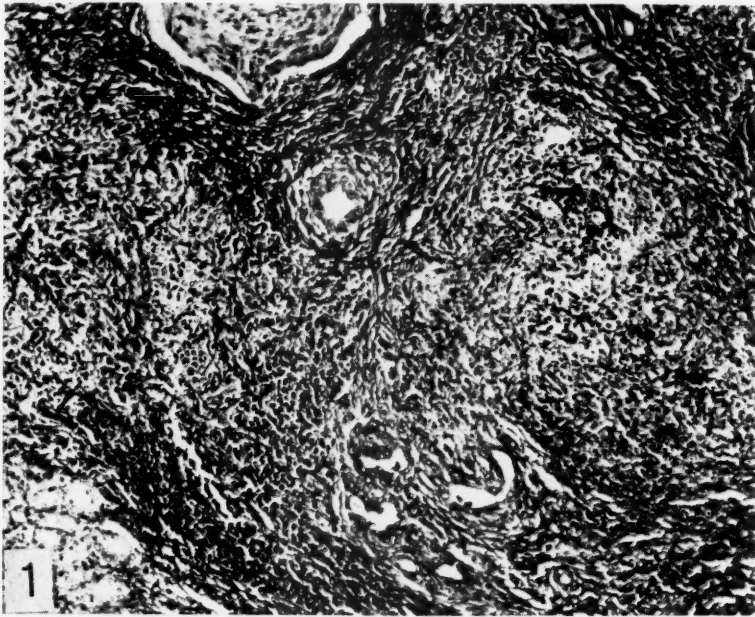
Fig. 2. — Guinea pig No. 21. Pancreas. Dense proliferation of metaplastic mucus-secreting glands is seen. Hematoxylin — van Gieson. Medium magnification.

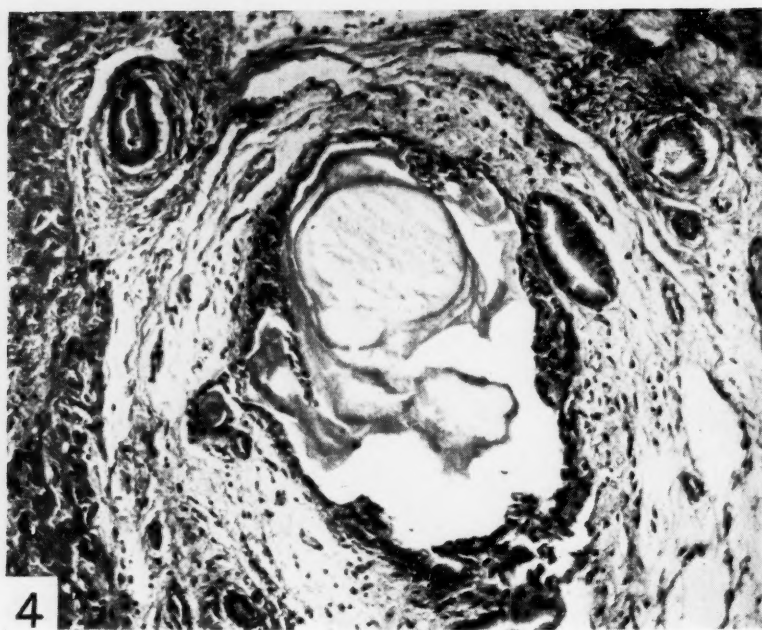
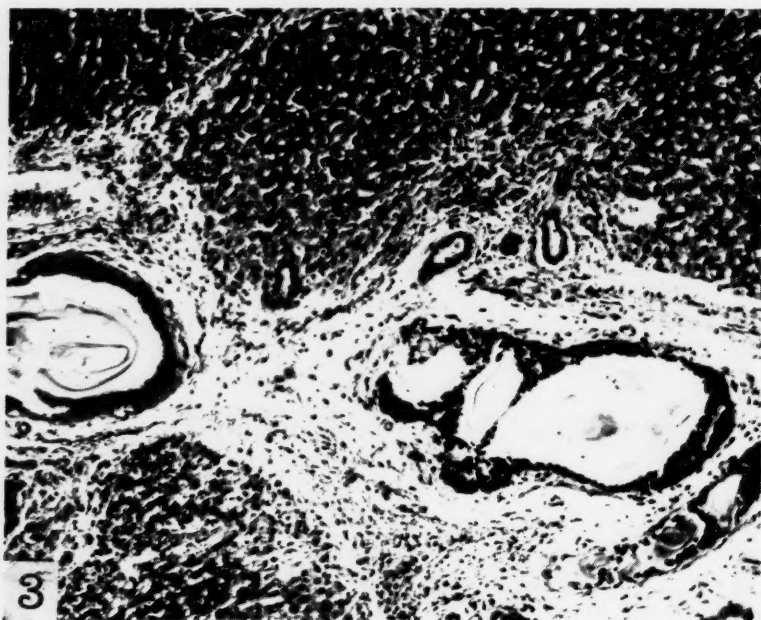
Fig. 3. — Rabbit No 32. Liver. The picture shows enlarged bile ducts and increase in the amount of surrounding connective tissue. Hematoxylin and eosin. Low-power magnification.

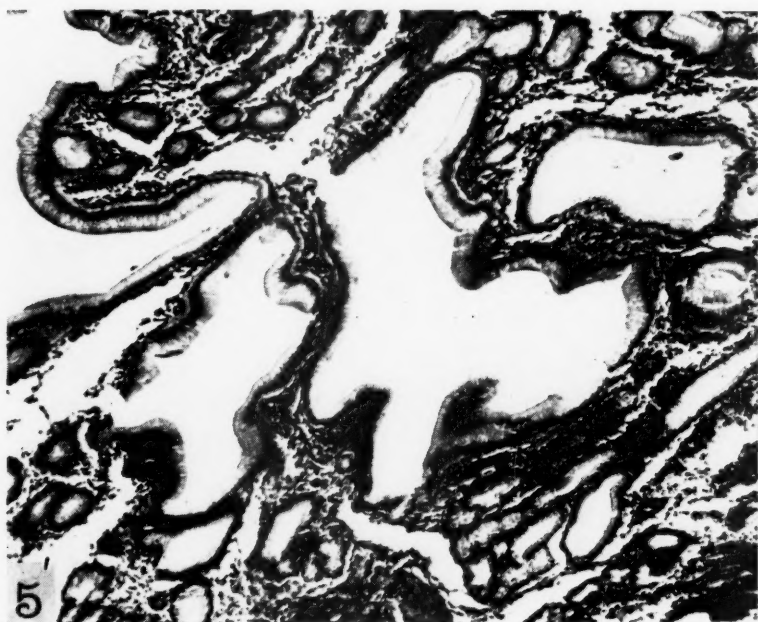
Fig. 4. — Rabbit No. 32. Liver. An enlarged bile duct containing eosinophilic lamellated material is seen. Hematoxylin and eosin. Medium magnification.

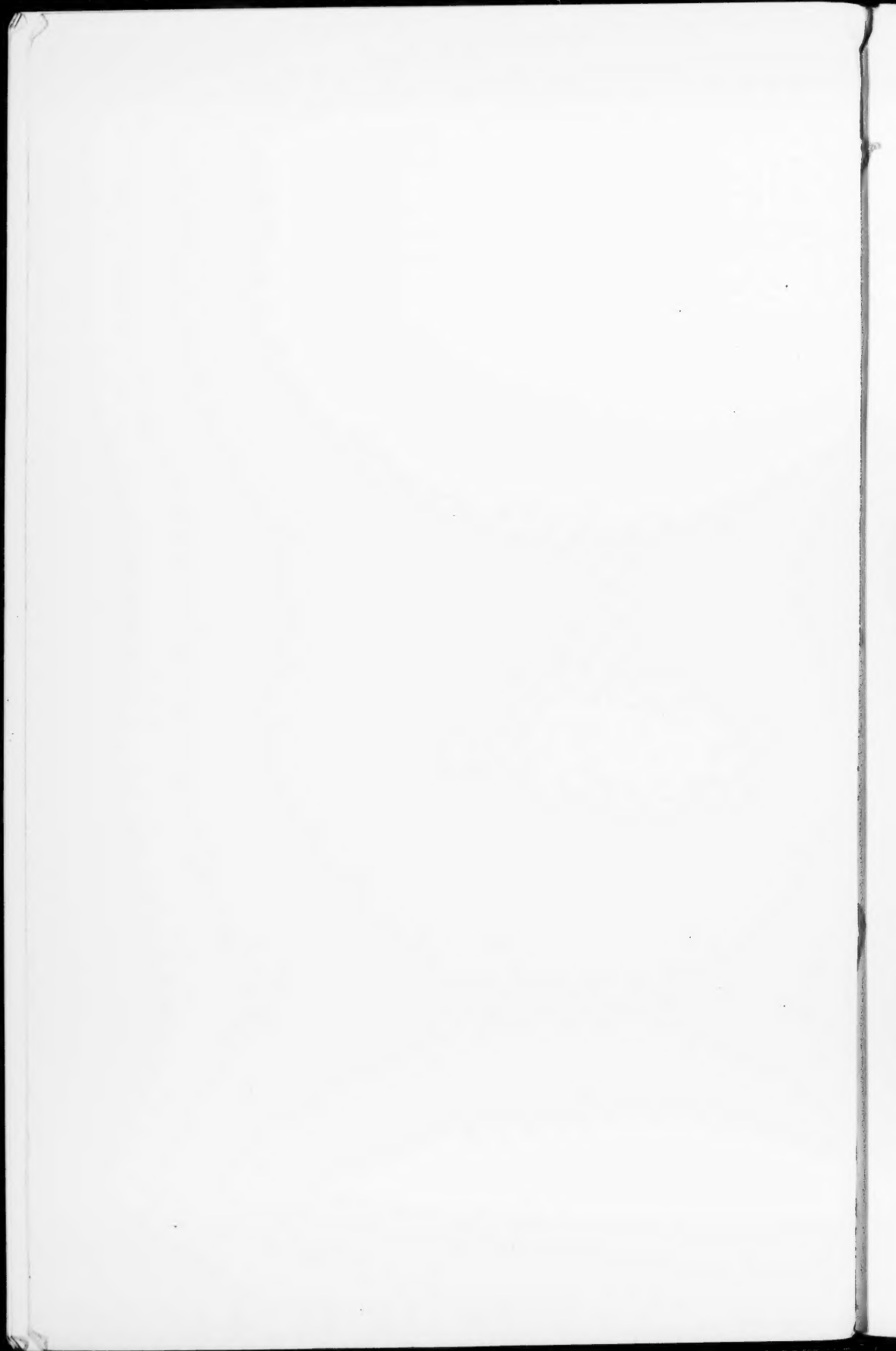
Fig. 5. — Rhesus monkey No. 15. Section from the corpus of the stomach. The principal glandular apparatus has disappeared and is replaced by proliferating, metaplastic, mucus-secreting cells (so called pseudopyloric metaplasia). Hematoxylin — van Gieson. Medium magnification.

Fig. 6. — Rat No. 5. Large intestine. The typical lining goblet cells are lacking. There are scattered meta- and anaplastic basophilic cells in one or several layers (especially in the left upper part of the picture). Hematoxylin — van Gieson. Low-power magnification.









# BARBAMYL

hypnoticum —  
sedativum



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